

Acid-induced aromatic hydrogen exchange. VI

Discussion of the kinetic isotope effect

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1. Introduction

The model advanced in Part V of this series [1] to describe the detailed mechanism of the electrophilic aromatic hydrogen exchange calls for some care in the interpretation of the kinetic isotope effect. The interrelation between the directions of the symmetry axes of the two carbon orbitals responsible for the carbon-hydrogen bonds introduces a coupling between the two corresponding angular motions. In the general case it will consequently hardly be possible to identify the reaction coordinate with a single-atom movement as has become the common usage in simple discussions of hydrogen isotope effects.

In the present paper an attempt is made to discuss the kinetic isotope effects of the hydrogen exchange in terms of one non-symmetrical and one symmetrical transition-state model, both of which are based on the mechanistic picture given in Part V [1].

2. Observed isotope effects

Table 1 shows isotope effects obtained in the replacement of heavy hydrogen isotopes from different aromatic positions by protons in acid media. The experimental errors are certainly large, and even if the isotope effect is not a smooth function of the reactivity of the position, it is quite evident that the trend shows that the isotope effect becomes stronger with increasing reactivity of the aromatic position.

Gold, Lambert, and Satchell [4] have also studied the solvent isotope effect of the detritiation of position 2 in 4-methylphenol in 4*N* aqueous hydrochloric acid at 25°C. The result was $k_{D_2O}/k_{H_2O} = 1.6_2$. According to a private communication, Dr. A. J. Kresge has obtained the value $k_{D_2O}/k_{H_2O} = 1.67$ in a similar study of the detritiation of 1,3,5-trimethoxybenzene in 0.05 *N* aqueous perchloric acid.

3. Non-symmetric transition-state model

As mentioned in Part V [1] many pieces of experimental evidence speak in favour of a two-step reaction mechanism for the aromatic hydrogen exchange. Two non-symmetric transition states will then have to be passed through, and

Table 1. Observed kinetic isotope effects in the exchange of aromatic tritium and deuterium against protium.

Compound	Position	Medium	Temp.	$k_{\text{ArT}}/k_{\text{ArD}}$	Reference
Benzene		80.7 % aq. H_2SO_4	25°C	0.64 ± 0.02	[2]
		87.1 % aq. H_2SO_4	25°C	0.59	M. Russell, prelim.
Toluene	<i>m</i>	80.7 % aq. H_2SO_4	25°C	0.64 ± 0.02	[2]
	<i>o</i>	80.7 % aq. H_2SO_4	25°C	0.56 ± 0.02	[2]
	<i>p</i>	80.7 % aq. H_2SO_4	25°C	0.52 ± 0.02	[2]
Thiophene	3	57.0 % aq. H_2SO_4	25°C	0.59 ± 0.04	[3]
	2	57.0 % aq. H_2SO_4	25°C	0.51 ± 0.03	[3]
Anisole	<i>o</i>	65.2 % aq. H_2SO_4	25°C	0.45	M. Russell, prelim.
	<i>p</i>	65.2 % aq. H_2SO_4	25°C	0.47	M. Russell, prelim.
4-Methylphenol	2	3.5–6.5 <i>N</i> aq. HCl	25°C	0.3–0.5	[4]
1,3,5-Trimethoxy-benzene		0.05 <i>N</i> aq. HClO_4		0.47	A. J. Kresge, priv. comm.

they will be mutual isotopic isomers. Their two carbon orbitals corresponding to the two carbon-hydrogen bonds will be in some state of the type illustrated by *b* or *d* in Fig. 6 of reference [1].

For a discussion of the isotope effect it is necessary to know something about the vibrational pattern of the transition state. Owing to the complexity of the problem, most approaches in similar cases come no further than to mere guesses. In the case of aromatic substitution, however, the conformation of the transition state is fairly fixed, at least roughly, in many of its details. Except for minor changes in the ring angles and carbon-carbon interatomic distances (cf. [1]) the geometrical changes in the relative positions of the nuclei are likely to embrace mainly the incoming substituent, the outgoing one, and the extra-annular orbitals of the carbon atom at the centre of reaction. In the case of the hydrogen exchange, the small masses of the extra-annular substituents make the situation particularly favourable for a simplified treatment.

In the following it will be assumed that the movement of the two hydrogens in that particular plane which is perpendicular to the ring plane and also contains the carbon atom concerned and the carbon atom in the *para* position, might be treated separately from the other nuclear movements, and that the former carbon atom and the plane are at rest. Consequently the movements of the hydrogens within this fixed framework may be considered as internal ones, and they could be treated by means of internal coordinates.

The plane in question is represented by that of the paper in Fig. 1. The indices *v* and *w* used in the following correspond to the notations of the angles and directions used in ref. [1], and we assume for the angles that $v > w$. r_v and r_w are the equilibrium carbon-hydrogen distances, and m_v and m_w are the masses of the hydrogen nuclei. ρ_v and ρ_w are the displacements in the bond directions, and $r_v \delta_v$ and $r_w \delta_w$ are the displacements perpendicular to these directions, δ_v and δ_w being small angular displacements. The deviations from the equilibrium positions are treated as positive when they have the direction indicated in Fig. 1.

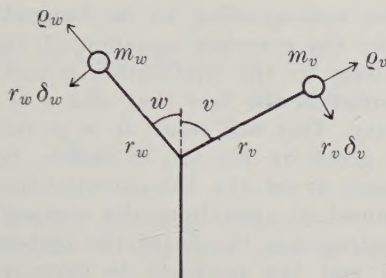


Fig. 1. Displacement coordinates and symbols used in the vibrational analysis of the nonsymmetric transition state.

The kinetic-energy function T is given by

$$2T = m_v \dot{Q}_v^2 + m_v r_v^2 \dot{\delta}_v^2 + m_w \dot{Q}_w^2 + m_w r_w^2 \dot{\delta}_w^2 \quad (1)$$

where the dots indicate the time derivatives as usual.

The difficult thing is to design a potential-energy function that is realistic enough. To make a quantum-mechanical calculation accurate enough for this function to be found, even to the second-degree approximation, is a formidable problem, as it allows no simple approximate separation into σ - and π -electron systems. On the other hand, the problem seems to be of such interest that it might be better to make at least a simple guessing approach than to dismiss it altogether. It could be hoped that the results, although not quantitatively valid, will show some principles of importance.

We shall first discuss the coupling between the two carbon-hydrogen bond directions. From Fig. 1 it is evident that the small angular deviations δ_v and δ_w could be considered as small changes in the angles v and w . The relation between v and w was found to be [1]

$$\cot v \cot w = 1 - \cot^2 u \quad (2)$$

where u was half the carbon-carbon-carbon angle in the ring. This relation is illustrated in Fig. 3 of ref. [1]. If u is treated as a constant, derivation of [2] gives

$$\frac{dw}{dv} = -\frac{1 - \cot^2 u}{1 - (2 - \cot^2 u) \cot^2 u \sin^2 v} = -p \quad (3)$$

For small deviations δ_v and δ_w no increase in potential energy arises as a result of the angular coupling if

$$\frac{\delta_w}{\delta_v} = \frac{\Delta w}{\Delta v} \approx \frac{dw}{dv} = -p$$

The best second-degree potential-energy function corresponding to this angular coupling is

$$V_{\delta_v \delta_w} = \frac{1}{2} k_{\delta_v \delta_w} \left(\sqrt{p} \delta_v + \frac{1}{\sqrt{p}} \delta_w \right)^2 \quad (4)$$

where $k_{\delta_v \delta_w}$ and p are positive constants. This function remains equal to zero as long as $\delta_w/\delta_v = -p$, i.e., as long as the angular deviations δ_v and δ_w are inter-

related by a linear equation corresponding to the tangent of the w, v curve (Fig. 3 of ref. [1]) rather than by the complete equation (2) itself. (The tangent is, of course, the one corresponding to the equilibrium w and v values.) There is a further approximation inherent in the fact that also u is likely to undergo some change when w and v vary. This will result in a p value somewhat different from the approximate one given by Eq. (3), cf. below, Section 5.

According to the discussion in ref. [1], the potential-energy maximum defining the transition state is assumed to arise from the competition between the tendency towards increased conjugation of one of the carbon orbitals with the remaining π -electron system and the tendency to improve the carbon-hydrogen bond corresponding to the same orbital. This orbital and bond corresponds to index v in Fig. 1. The other orbital or bond was assumed to be more indifferent in these respects, as it is closer to the ring plane and should altogether be a fairly stable carbon-hydrogen bond. We shall try to reproduce these effects by the very simple potential-energy term

$$V_{\delta v} = -\frac{1}{2} k_{\delta v} \delta_v^2 \quad (5)$$

where $k_{\delta v}$ is a positive constant for positions of low reactivity. This is a rough approximation, as the value of δ_w is certainly not immaterial, and the use of (5) involves some confusion between the angular position of the hydrogen nucleus and that of the carbon orbital.

It is evident that a movement of hydrogen v in the direction of positive δ_v should be accompanied by some lengthening of the equilibrium carbon-hydrogen bond distance. This is accounted for by the inclusion of a cross-term

$$V_{qv\delta v} = -k_{qv\delta v} q_v \delta_v \quad (6)$$

where $k_{qv\delta v}$ is a positive constant. This term is certainly subject to the same objections as $V_{\delta v}$.

With two more terms, accounting for the stretching force constants of the two bonds, the total potential-energy function V is given by

$$2V = k_{qv} q_v^2 - k_{\delta v} \delta_v^2 + k_{qw} q_w^2 + k_{\delta v \delta w} \left(\sqrt{p} \delta_v + \frac{1}{\sqrt{p}} \delta_w \right)^2 - 2k_{qv\delta v} q_v \delta_v \quad (7)$$

k_{qv} and k_{qw} are, of course, positive constants. The other parameters have been defined above.

Some of the influence from δ_w on the effects expressed by the simple Eqs. (5) and (6) can certainly be considered as included in the term given by Eq. (4) if the two parameters of the latter are properly adjusted. In such a case this will have to be remembered in the discussion of their probable values. It is believed, however, that nothing essential is changed in the following discussion by such a modification of their meaning. A more general potential-energy function will be more or less useless owing to the difficulty in assessing the different magnitudes.

Eqs. (1) and (7) lead to the following secular equation:

$$\begin{vmatrix} k_{qv} - m_v \lambda & -k_{qv\delta v} & 0 & 0 \\ -k_{qv\delta v} & -k_{\delta v} + p k_{\delta v\delta w} - m_v r_v^2 \lambda & 0 & k_{\delta v\delta w} \\ 0 & 0 & k_{qw} - m_w \lambda & 0 \\ 0 & k_{\delta v\delta w} & 0 & \frac{1}{p} k_{\delta v\delta w} - m_w r_w^2 \lambda \end{vmatrix} = 0 \quad (8)$$

where, as usual, $\lambda = 4\pi^2 \nu^2$, ν denoting frequency.

The stretching movement of hydrogen atom w separates out at once, giving

$$\lambda_4 = k_{qw} / m_w \quad (9)$$

The other three frequencies are determined by

$$\lambda_1 + \lambda_2 + \lambda_3 = \frac{k_{qv}}{m_v} + \frac{p k_{\delta v\delta w} - k_{\delta v}}{m_v r_v^2} + \frac{\frac{1}{p} k_{\delta v\delta w}}{m_w r_w^2} \quad (10)$$

$$\lambda_1 \lambda_2 + \lambda_2 \lambda_3 + \lambda_1 \lambda_3 = \frac{p k_{qv} k_{\delta v\delta w} - k_{qv} k_{\delta v} - k_{qv\delta v}^2}{m_v^2 r_v^2} + \frac{\frac{1}{p} k_{qv} k_{\delta v\delta w}}{m_v m_w r_w^2} - \frac{\frac{1}{p} k_{\delta v} k_{\delta v\delta w}}{m_v m_w r_v^2 r_w^2} \quad (11)$$

$$\lambda_1 \lambda_2 \lambda_3 = -\frac{\frac{1}{p} k_{\delta v\delta w} (k_{qv} k_{\delta v} + k_{qv\delta v}^2)}{m_v^2 m_w r_v^2 r_w^2} \quad (12)$$

These expressions might seem somewhat useless at first sight, since the different parameters are little known. One way to introduce some simplification is the following. Since all parameters k and also p are positive, it is obvious from Eq. (12) that there must exist a negative root, say λ_1 . It corresponds, of course, to the movement along the reaction coordinate, which has an imaginary frequency. Let us assume that λ_1 has a very small absolute value of negative sign, i.e. that the potential-energy surface has a considerably smaller curvature along the reaction coordinate than in other directions, an assumption that is not too unnatural. Since $k_{\delta v\delta w}$ is likely to have an appreciable value, it can be seen from Eq. (12) that our assumption requires $k_{qv\delta v}$, and at least one of k_{qv} and $k_{\delta v}$, to be small. If the small quantities are neglected in comparison with the others, we obtain the following approximate equations for λ_2 and λ_3 from Eqs. (10 and (11):

$$\lambda_2 + \lambda_3 = \frac{k_{qv}}{m_v} + \frac{p k_{\delta v\delta w} - k_{\delta v}}{m_v r_v^2} + \frac{\frac{1}{p} k_{\delta v\delta w}}{m_w r_w^2} \quad (13)$$

$$\lambda_2 \lambda_3 = \frac{p k_{qv} k_{\delta v\delta w}}{m_v^2 r_v^2} + \frac{\frac{1}{p} k_{qv} k_{\delta v\delta w}}{m_v m_w r_w^2} - \frac{\frac{1}{p} k_{\delta v} k_{\delta v\delta w}}{m_v m_w r_v^2 r_w^2} \quad (14)$$

These equations might be simplified further when it has been decided which one of the two parameters k_{qv} and $k_{\delta v}$ should be assigned a vanishing value.

Already at the present stage it is possible to obtain an approximate but general expression telling the ratio of the reduced masses or the square of the ratio of the frequencies for the movement along the reaction coordinate in two isotopic cases. We have by means of Eqs. (12) and (14)

$$\frac{m^{*''}}{m^{*'}} = \left(\frac{\nu_L^{\dagger'}}{\nu_L^{\dagger''}} \right)^2 = \frac{\lambda_1'}{\lambda_1''} = \frac{\lambda_1' \lambda_2' \lambda_3'}{\lambda_1'' \lambda_2'' \lambda_3''} \cdot \frac{\lambda_2'' \lambda_3''}{\lambda_2' \lambda_3'} = \frac{k_{qv} \left(\frac{1}{p} m_v'' r_v^2 + p m_w'' r_w^2 \right) - \frac{1}{p} k_{\delta v} m_v''}{k_{qv} \left(\frac{1}{p} m_v' r_v^2 + p m_w' r_w^2 \right) - \frac{1}{p} k_{\delta v} m_v'} \quad (15)$$

where m^* denotes the reduced mass along the reaction coordinate, $\nu_L^{\dagger}$ the corresponding (imaginary) frequency, and prime and bis denote two different isotopic cases.

From this point it is convenient to study two different limiting situations. From the discussion [1] of the mechanism of the reaction it was found that there is a gradual shift in the nature of the transition state with changing reactivity of the aromatic position concerned. With increasing reactivity the transition state will change in the direction from the stable intermediate towards the π -complex. This means probably also that the movement along the reaction coordinate shifts from a mainly bending motion of the two hydrogens towards a mainly stretching motion of one of the carbon-hydrogen bonds.

From Eq. (15) it is evident that, if we assume $k_{\delta v} \ll k_{qv} r^2$ (r is a mean value of r_v and r_w , and p is assumed to be of the order of unity), we obtain

$$\frac{m^{*''}}{m^{*'}} = \frac{\frac{1}{p} m_v'' r_v^2 + p m_w'' r_w^2}{\frac{1}{p} m_v' r_v^2 + p m_w' r_w^2} = \frac{\frac{1}{p} I_v'' + p I_w''}{\frac{1}{p} I_v' + p I_w'} \quad (16)$$

which indicates a combined bending motion (I denotes moment of inertia).

On the other hand, if we assume $k_{\delta v} \gg k_{qv} r^2$ we obtain

$$\frac{m^{*''}}{m^{*'}} = \frac{m_v''}{m_v'} \quad (17)$$

which indicates a translation of the single atom v .

Low reactivity. The limiting situation of very low reactivity can be reproduced by our model if we assign very small values to $k_{qv\delta v}$ and $k_{\delta v}$ but let k_{qv} be of ordinary magnitude. Close to the intermediate the lengthening of the bond with increasing δ_v is probably small, and the smallness of $k_{\delta v}$ is a consequence of our assumption of a small curvature of the potential-energy surface along the reaction coordinate. Thus, nothing unnatural is inherent in our assignments. The equations (13) and (14) are now simplified to the following approximate ones:

$$\lambda_2 + \lambda_3 = \frac{k_{qv}}{m_v} + \frac{p k_{\delta v \delta w}}{m_v r_v^2} + \frac{\frac{1}{p} k_{\delta v \delta w}}{m_w r_w^2}$$

$$\lambda_2 \lambda_3 = \frac{k_{\varrho v}}{m_v} \left(\frac{p k_{\delta v \delta w}}{m_v r_v^2} + \frac{1}{p} \frac{k_{\delta v \delta w}}{m_w r_w^2} \right)$$

or

$$\lambda_2 = k_{\delta v \delta w} \left(\frac{1}{\frac{1}{p} m_v r_v^2} + \frac{1}{p m_w r_w^2} \right) \quad (18)$$

$$\lambda_3 = \frac{k_{\varrho v}}{m_v} \quad (19)$$

The approximations introduced make it possible to express in simple terms the frequencies of the four normal modes of vibration of our transition-state model. The frequencies of the truly periodic motions could be found from Eqs. (18), (19), and (9), the ratio of the reduced masses along the decomposition coordinate for two different isotopic cases is given by Eq. (16), and the form of the respective normal vibrations can be found by means of Eq. (8). Fig. 2 gives a survey. It is obvious that the angular modes of vibration correspond to the "rocking" and "scissoring" vibrations of methylene groups observed for instance in long-chain normal paraffins [5].

The interesting thing about the vibrational pattern of Fig. 2 is the symmetry in the masses m_v and m_w . If $r_v/r_w = p$, i.e., $r_v^2/p = p r_w^2$, and also $k_{\varrho v} = k_{\varrho w}$, the sum of the real frequencies and also the reduced mass ratio along the reaction coordinate is invariant on interchanging the two hydrogen masses m_v and m_w .

High reactivity. With highly reactive aromatic positions it is probable that the transition state is reached already with the transfer of the hydrogen nucleus from some base [1]. This situation is much more difficult to treat theoretically. The mere fact that we are faced with a three-centre reaction is likely to introduce complications which cannot be handled with confidence [6a]. It is also hard to assess the relative magnitudes of the force constants, but in the following the previous general scheme will be applied with the assumptions that $k_{\varrho v}$ is equal to zero, that $k_{\varrho v \delta v}$ is small, and that $k_{\delta v}$ has a finite negative value. The transition state has more the character of a π -complex, and the hydrogen transfer occurs in the carbon-hydrogen direction. The negative $k_{\delta v}$ corresponds to a force opposing bending movements of the same hydrogen. The approximate Eqs. (13) and (14) give

$$\lambda_2 + \lambda_3 = \frac{p k_{\delta v \delta w} - k_{\delta v}}{m_v r_v^2} + \frac{1}{p} \frac{k_{\delta v \delta w}}{m_w r_w^2} \quad (20)$$

$$\lambda_2 \lambda_3 = - \frac{\frac{1}{p} k_{\delta v} k_{\delta v \delta w}}{m_v m_w r_v^2 r_w^2} \quad (21)$$

and Eq. (17) gives the ratio of the reduced masses along the reaction coordinate for two different isotopic cases. Obviously the two vibrational modes corresponding to (20) and (21) are ordinary periodic bending motions.

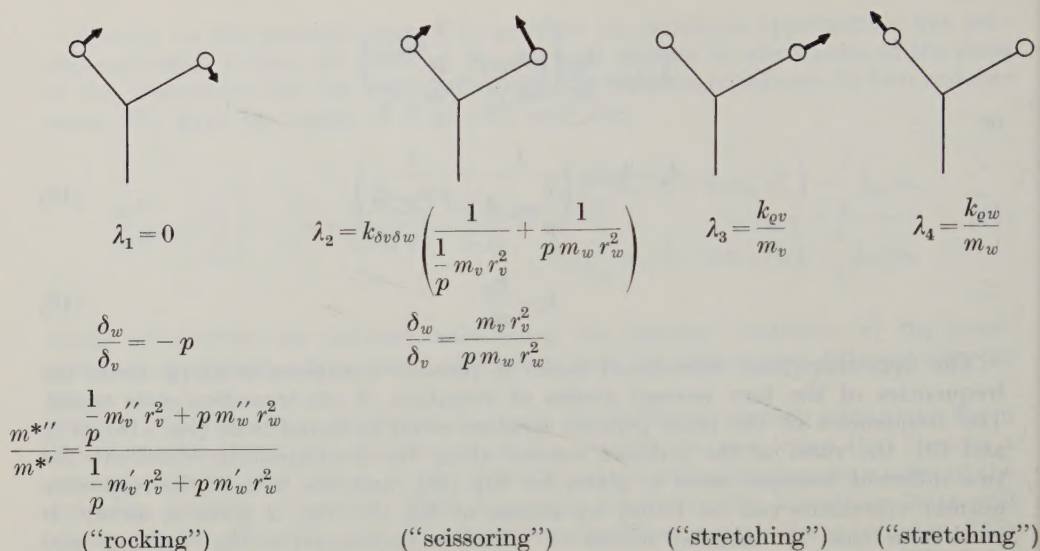


Fig. 2. Limiting normal modes of vibration of non-symmetric transition state at low reactivity.

The symmetry between m_v and m_w observed at the limit of low reactivity does not exist in this case.

4. Symmetric transition-state model

A symmetric type of transition state, i.e., a single-step reaction mechanism for the hydrogen exchange, does not seem likely for several experimental reasons [1]. For the sake of completeness, however, the vibrational behaviour of a very simple model also of that kind will be treated in this connection. It will be seen that the characteristic bending behaviour of the low-reactivity model of Section 3 is also shown by a similar model with a potential that is symmetric in the two angles δ_v and δ_w .

In the following it will be assumed that there is no appreciable coupling between the radial and the angular motions, and only the latter will be treated. According to ref. [1] the motion along the reaction coordinate will to an appreciable extent be a rotational (bending) one for not too highly reactive aromatic positions, and this will be the case particularly in the vicinity of the symmetrical intermediate, which will be able to offer two carbon-hydrogen bonds of approximately sp^3 character.

Two competing influences could be imagined to be acting. The angular coupling between the two carbon orbitals tends to keep the two bonds at some particular mutual angle, not far from the tetrahedral one. On the other hand, there is energy to gain if the aromatic conjugation could be restored, and there will thus be forces tending to move either hydrogen in the direction away from the ring plane. A corresponding spring model would be strained in its equilibrium conformation; the spring tending to keep the "natural" angle between the two carbon-hydrogen bonds would be somewhat stretched by two other springs, each of which tends to increase the angle between one of the bonds and the ring plane.

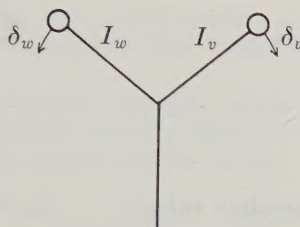


Fig. 3. Angular displacement coordinates and moments of inertia used in the vibrational analysis of the symmetric transition state.

Fig. 3 shows the two angular displacement coordinates δ_v and δ_w . A suitable potential-energy function, V , is given by

$$2V = (k_\alpha - k_\beta)(\delta_v^2 + \delta_w^2) + 2k_\alpha \delta_v \delta_w \quad (22)$$

where k_α and k_β are positive constants. The physical meaning of Eq. (22) is best seen if we look at the angular momentum acting on atom v in the positive δ_v direction,

$$-\frac{\partial V}{\partial \delta_v} = k_\beta \delta_v - k_\alpha (\delta_v + \delta_w) \quad (23)$$

It can be considered to be made up by one term, $-k_\alpha (\delta_v + \delta_w) - k_0$, representing the action of the first-mentioned spring, and another term, $k_\beta \delta_v + k_0$, representing that of one of the latter springs. The two constant moments, $-k_0$ and $+k_0$, operate at equilibrium but balance one another. They need not be known as they do not appear in the vibrational analysis. Since the equilibrium conformation is the symmetric one there is no need for the parameter p appearing in the treatment in Section 3, or $p=1$.

The kinetic-energy function T is simply determined by

$$2T = I_v \dot{\delta}_v^2 + I_w \dot{\delta}_w^2 \quad (24)$$

where I denotes the moment of inertia. Eqs. (22) and (24) give the following secular equation:

$$\begin{vmatrix} k_\alpha - k_\beta - I_v \lambda & k_\alpha \\ k_\alpha & k_\alpha - k_\beta - I_w \lambda \end{vmatrix} = 0 \quad (25)$$

where, as usual, $\lambda = 4\pi^2 \nu^2$, ν being the frequency. We obtain

$$\lambda_1 + \lambda_2 = (k_\alpha - k_\beta) \left(\frac{1}{I_v} + \frac{1}{I_w} \right) \quad (26)$$

$$\lambda_1 \lambda_2 = \frac{k_\beta (k_\beta - 2k_\alpha)}{I_v I_w} \quad (27)$$

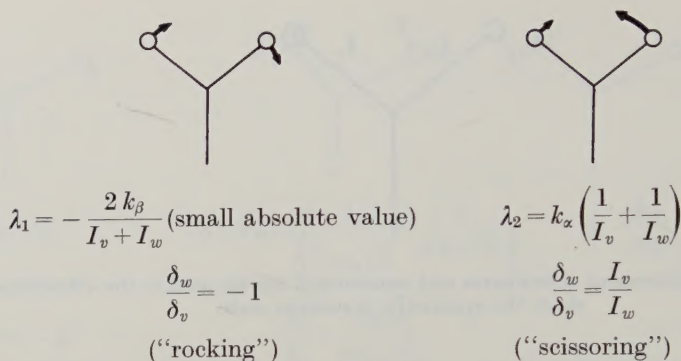


Fig. 4. Limiting normal modes of vibration of symmetric transition state.

Already from (22) it can be seen that the potential-energy surface corresponds to a saddle point around $\delta_v = 0$, $\delta_w = 0$ if $2k_\alpha > k_\beta$. From (26) and (27) it is evident that we will obtain one large real frequency and one small imaginary one as desired if k_β is chosen small in comparison to k_α . At the limit $k_\alpha \gg k_\beta$ we obtain approximately

$$\lambda_1 = -\frac{2k_\beta}{I_v + I_w} \quad (28)$$

$$\lambda_2 = k_\alpha \left(\frac{1}{I_v} + \frac{1}{I_w} \right) \quad (29)$$

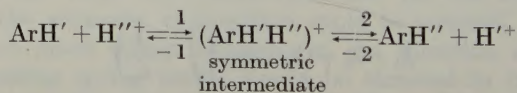
The form of the corresponding normal modes of vibration is shown in the survey of Fig. 4. It is evident that these two modes are closely analogous to the "rocking" and "scissoring" modes of the non-symmetric low-reactivity model, cf. Fig. 2.

5. Discussion

The simple limiting models discussed in Sections 3 and 4 have been studied in order to show the outlines of the behaviour of transition states in which the reaction coordinate embraces more than one single bond. It is not believed that any real transition state will be very close to, for instance, the model of Fig. 2, but the conclusions drawn from that model might nevertheless be helpful in a semi-quantitative discussion of the behaviour of less reactive aromatic positions.

(a) Two-stage reaction

The hydrogen exchange is very likely, from all points of view, to proceed via a two-step mechanism which could be written schematically



where H' and H'' are two different hydrogen atoms which should be isotopic for the reaction to be observable. The reactions 1 and -1 on the one hand and

2 and -2 on the other pass two non-symmetric transition states which are isotopic isomers but otherwise identical. The model of Section 3 will be used for a discussion of them.

The specific rate of the forward reaction, which is generally determined from the initial rate and the observed isotope effect of which is given in Table 1, is found from the steady-state approximation to be

$$k = \frac{k_1 k_2}{k_{-1} + k_2} = \frac{k_1}{\frac{k_{-1}}{k_2} + 1}$$

The fraction in the denominator is the ratio between the rates of two analogous, competing reaction paths of the intermediate, in the observable cases an intramolecular isotope effect.

In all experiments listed in Table 1, H' is a heavy hydrogen isotope, given by the index of k , and H'' is protium. The observed isotope effects can obviously be written

$$\frac{k_{\text{ArT}}}{k_{\text{ArD}}} = \frac{k_{1(\text{ArT})}}{k_{1(\text{ArD})}} \cdot \frac{\left(\frac{k_{\text{H}}}{k_{\text{D}}}\right)_{\text{intra}} + 1}{\left(\frac{k_{\text{H}}}{k_{\text{T}}}\right)_{\text{intra}} + 1} \quad (30)$$

where the fractions in brackets, indexed "intra", are the intramolecular isotope effects for the decompositions of the intermediates. (The indices of these k 's give the kind of atom split off.)

The deuterium solvent isotope effect of detritiation has been measured in two cases referred to in Section 2. In these cases H' is tritium and H'' is deuterium or protium. The observed rate ratio could be written

$$\frac{k_{\text{D}_2\text{O}}}{k_{\text{H}_2\text{O}}} = \frac{k_{1(\text{D}_2\text{O})}}{k_{1(\text{H}_2\text{O})}} \cdot \frac{\left(\frac{k_{\text{H}}}{k_{\text{T}}}\right)_{\text{intra}} + 1}{\left(\frac{k_{\text{D}}}{k_{\text{T}}}\right)_{\text{intra}} + 1} \quad (31)$$

and it should be remembered for the k_1 ratio that tritium is present initially in the aromatic position.

Low reactivity. As shown in Section 3, the sum of the three real frequencies of the transition state "at low reactivity" is completely invariant for an interchange of the hydrogen masses m_v and m_w , provided that $r_v/r_w = p$ and $k_{\text{ov}} = k_{\text{ow}}$. From Eq. (3) it is found that, for $v > w$, p varies between 1 and 3/2 for $u = 60^\circ$ and between 1 and 2 for u equal to half the tetrahedral angle. In reality u probably varies simultaneously, which might make the v, w curve somewhat steeper than indicated by Fig. 3 of ref. [1] for some part of the same interval. For the equilibrium conformation of the symmetric intermediate itself, however, p must obviously be unity because of the symmetry. Since we are probably not far from the conformation of the intermediate, v is only slightly larger than w and $p = -dw/dv$ could be expected to be only slightly larger than unity. Since the bond making the largest angle with the plane of the ring has also the smallest

s character, it is also probable that r_v is somewhat larger than r_w . Hence the condition $r_v/r_w = p$ is likely to be approximately fulfilled. The carbon-hydrogen stretching force constant does not vary too rapidly with the fractional s character [7], and thus also the condition $k_{ev} = k_{ew}$ is approximately satisfied.

Any intramolecular isotope effect is a function only of the properties of the transition states, as all data about the common reactant are cancelled out from the specific rate ratio. Since we are dealing with isotopic hydrogen in a heavy molecule, it is permissible to write this isotope effect as a pure zero-point energy effect [6b] and we have, according to Eq. (2-9) of ref. [6] (with $u = h\nu/kT$)

$$\left(\frac{k_{H''}}{k_{H'}}\right)_{\text{intra}} = \exp \left\{ -\frac{1}{2kT} \sum_i^{3n^{\ddagger}-7} (\nu_{i(H'')}^{\ddagger} - \nu_{i(H')}^{\ddagger}) \right\} \approx \exp \left\{ -\frac{1}{2kT} \left[\sum_i^3 \nu_{i(H'')}^{\ddagger} - \sum_i^3 \nu_{i(H')}^{\ddagger} \right] \right\} \approx 1$$

(h is Planck's constant, k is Boltzmann's constant, T is the absolute temperature, $n^{\ddagger}$ is the number of atoms in the transition state, and the sign $\ddagger$ is the transition-state symbol.) The three-membered sums include the three periodic modes discussed. (The two carbon-hydrogen bendings not included in our model are assumed to have the same frequency irrespective of whether the hydrogen atom plays the role denoted by index v or w in the model of Section 3, hence they disappear by cancellation.) All other modes of vibration are assumed to be non-isotopic.

It is also interesting to note that the ratio of the reduced masses along the reaction coordinate is unity under the above assumptions. This can be seen from Eq. (16) by inserting $m_v'' = m_w''$ and $m_w'' = m_v$.

It follows that we can write, for the low-reactivity model, according to (30) and (31), respectively,

$$\frac{k_{\text{ArT}}}{k_{\text{ArD}}} = \frac{k_{1(\text{ArT})}}{k_{1(\text{ArD})}} \quad \text{and} \quad \frac{k_{\text{D}_2\text{O}}}{k_{\text{H}_2\text{O}}} = \frac{k_{1(\text{D}_2\text{O})}}{k_{1(\text{H}_2\text{O})}}$$

i.e., the observed isotope effect represents that of step 1.

In all experiments referred to in Table 1, the transition state of reaction 1 carries the heavy atom in the position denoted by index w in Section 3 and a protium atom in the position denoted by index v . If we apply Eq. (2-9) of ref. [6] also to this situation, we have to write out in full ($u = h\nu/kT$)

$$\frac{k_{1(\text{ArT})}}{k_{1(\text{ArD})}} = \exp \left\{ -\frac{1}{2kT} \left[\sum_i^{3n^{\ddagger}-7} (\nu_{i(\text{T})}^{\ddagger} - \nu_{i(\text{D})}^{\ddagger}) - \sum_i^{3n-6} (\nu_{i(\text{T})} - \nu_{i(\text{D})}) \right] \right\}$$

(n denotes the number of atoms in the isotopic reactant.) Only three frequencies in the reactant and five of the real ones in the transition state will be appreciably sensitive to the two hydrogen masses concerned. All other frequencies of the two sums will disappear by cancellation. The stretching of the protium bond in the transition state corresponds to λ_3 (Fig. 2) and is consequently independent of the other hydrogen modes. It has the same frequency in both transition states and could be left out from the first sum. The same applies to that bending of the same bond which has not been included in our model. We are now left with three terms in the first sum and three in the second sum. It seems fair to assume cancellation between each transition state and the corresponding reactant for the

stretching of the isotopic bond, and, in the same way, between the bending-in-plane frequency of the reactant and that bending mode of the same bond in the transition state which has not been included in our model. What finally remains is the "scissoring" mode in the transition state and the bending-out-of-plane in the reactant. If these two frequencies (in wave number units) are denoted by $\bar{\nu}_s^+$ and $\bar{\nu}_b$, respectively, it is now possible to write (c is the velocity of light)

$$\frac{k_{1(\text{ArT})}}{k_{1(\text{ArD})}} = \exp \left\{ -\frac{1}{2} \frac{hc}{kT} \left[(\bar{\nu}_{s(\text{T})}^+ - \bar{\nu}_{s(\text{D})}^+) - (\bar{\nu}_{b(\text{T})} - \bar{\nu}_{b(\text{D})}) \right] \right\}$$

The out-of-plane frequency is generally of the order of 700 cm^{-1} for protium, and hence approximately

$$\bar{\nu}_{b(\text{T})} - \bar{\nu}_{b(\text{D})} = \left(\frac{1}{\sqrt{3}} - \frac{1}{\sqrt{2}} \right) \times 700 = -91 \text{ cm}^{-1}$$

If, for the moment, we assume that the transition-state difference does not compensate for this difference appreciably, the rate ratio would be $k_{1(\text{ArT})}/k_{1(\text{ArD})} = 0.80$ at 25°C . This is the strongest isotope effect for the couple tritium-deuterium compatible with the low out-of-plane frequency.

The difference corresponding to the transition state is difficult to estimate. If we assume that Eq. (18) should also be able to reproduce the scissoring frequency of ordinary long-chain paraffins (about 1500 cm^{-1} [5]) with the same value of $k_{\phi v \phi w}$, and if we put all distances r equal and $p=1$, the following is obtained:

$$\frac{\bar{\nu}_{s(\text{T})}^+}{1500} = \left(\frac{1 - \frac{1}{3}}{1 - \frac{1}{1}} \right)^{\frac{1}{2}}; \quad \frac{\bar{\nu}_{s(\text{D})}^+}{1500} = \left(\frac{1 - \frac{1}{2}}{1 - \frac{1}{1}} \right)^{\frac{1}{2}}$$

or
$$\bar{\nu}_{s(\text{T})}^+ = 1220 \text{ cm}^{-1}; \quad \bar{\nu}_{s(\text{D})}^+ = 1300 \text{ cm}^{-1}$$

i.e.,
$$\bar{\nu}_{s(\text{T})}^+ - \bar{\nu}_{s(\text{D})}^+ = -80 \text{ cm}^{-1}$$

This estimate is, of course, very crude. It seems, however, that we could expect an isotope effect much weaker than the above value for the first step and consequently for the whole reaction of the type concerned.

It is evident that the low-reactivity model predicts $k_{\text{ArT}}/k_{\text{ArD}}$ values well above those observed for the least reactive positions studied. In order to explain the experimental values we have to include a good deal of stretching in the reaction coordinate. The low-reactivity model indicates a way, however, by which the isotope effect may vanish, more or less, for aromatic positions of low reactivity.

No solvent isotope effects have been observed for the hydrogen exchange of little reactive aromatic positions. The isotope effect of the first step, which is decisive, depends as to direction and strength on the difference in sensitivity to the isotopic mass between the zero-point energy of the transition state and that of the reacting acid molecule (e.g. H_3O^+). Also in the transition state there are three modes which are sensitive, the stretching, the bending mode not included in our model, and the scissoring mode (cf. Fig. 2).

High reactivity. As pointed out already in Section 3, the situation is much more

complicated with highly reactive aromatic positions. It will not be attempted here to discuss the isotope effect from the three-centre point of view. Only a few general conclusions will be drawn.

The fact that one of the hydrogens in the transition state has a normal carbon-hydrogen stretching frequency, while the stretching mode of the other has been converted into the reaction coordinate, is enough to give rise to a considerable intramolecular isotope effect for the decomposition of the intermediate.

In the first step of reactions of the kind referred to in Table 1 non-isotopic hydrogen is transferred. The isotopic hydrogen atom occupies about the same position in the transition state as in the reactant. Eqs. (20) and (21) do not give simple explicit expressions for the roots λ_2 and λ_3 . Although the situation is not perspicuous, it seems likely that the zero-point energy difference that exists between the two isotopic reactants should largely survive in the transition state. Hence the first reaction step could be expected to show no appreciable isotope effect, and we can thus probably write in this case

$$\frac{k_{\text{ArT}}}{k_{\text{ArD}}} \approx \frac{\left(\frac{k_{\text{H}}}{k_{\text{D}}}\right)_{\text{intra}} + 1}{\left(\frac{k_{\text{H}}}{k_{\text{T}}}\right)_{\text{intra}} + 1}$$

In order to obtain an idea of the intramolecular isotope effects from the observed over-all effect, it is probably allowable to interrelate the former two effects according to Swain *et al.* [8] as tried by Olsson [2] in the case of benzene and toluene. (The positions in these molecules belong, however, to the least reactive ones hitherto studied.) The interrelation requires an independent vibrational pattern for each hydrogen, but the intermixing (cf. Eqs. (20) and (21)) is not likely to cause too serious deviations.

According to Table 1 an observed value of $k_{\text{ArT}}/k_{\text{ArD}} = 0.47$ seems to be representative of the most reactive positions studied. The treatment mentioned gives from this

$$\left(\frac{k_{\text{H}}}{k_{\text{D}}}\right)_{\text{intra}} \approx 6.6; \quad \left(\frac{k_{\text{H}}}{k_{\text{T}}}\right)_{\text{intra}} \approx \left(\frac{k_{\text{H}}}{k_{\text{D}}}\right)_{\text{intra}}^{1.44} \approx 15$$

which are strong but reasonable isotope effects at 25°C. Olsson's computations for benzene gave 3.4 and 5.9, respectively, which are undoubtedly weak.

For the solvent isotope effect of detritiation, we have to consider the full expression (31). In the transition state of the first step a deuteron or a proton is transferred and has lost at least some of the zero-point energy associated with stretching. Such transfers generally involve some normal isotope effect.

If we accept the above values for the intramolecular isotope effects and the concept of independent vibrations of the two hydrogens, the last fraction of Eq. (31) would be

$$\frac{\left(\frac{k_{\text{H}}}{k_{\text{T}}}\right)_{\text{intra}} + 1}{\left(\frac{k_{\text{D}}}{k_{\text{T}}}\right)_{\text{intra}} + 1} \approx \frac{15 + 1}{(15)^{0.31} + 1} \approx 5$$

The reasonable value $k_{1(\text{D}_2\text{O})}/k_{1(\text{H}_2\text{O})} = 1/3$ would give the total $k_{\text{D}_2\text{O}}/k_{\text{H}_2\text{O}} \approx 5/3$, which agrees very well with the results referred to in Section 2.

(b) *Single-stage reaction*

If there is no depression at the centre of the potential-energy curve, a possibility that seems rather unlikely to-day, the single transition state must be symmetric in the two hydrogens.

The symmetric transition-state model of Section 4 shows, in principle, the same mechanical behaviour as the non-symmetric low-reactivity model of Section 3. Both have the "rocking" mode, a pure bending motion, as the reaction coordinate. It follows that the conclusions drawn for k_1 of the two-stage low-reactivity reaction, are valid also for the specific rate of a single-step reaction corresponding to a transition state of the type of Section 4. Hence, in order to explain the effects listed in Table 1, we shall have to ascribe a good deal of stretching character to the reaction coordinate also in this case, because the frequencies and zero-point energies associated with stretching are larger than those with bending modes.

As explained below, the simultaneous assumption of a single-stage reaction and considerable stretching character of the reaction coordinate involves some difficulties with respect to other electrophilic substitution reactions. This fact tends to lay stress upon the unlikelihood of the single-stage mechanism.

(c) *Conclusions. Relation to other electrophilic aromatic substitution reactions*

The fact that the observed exchange reactions in which heavy isotopes of hydrogen in aromatic positions are exchanged for protium from an acid medium show isotope effects of the order of $k_{\text{ArT}}/k_{\text{ArD}} = 0.4$ to 0.7 at 25°C and that the least reactive positions show the weakest isotope effects seems at present to be best explained by a two-stage mechanism, account being taken of an angular coupling between the two carbon-hydrogen bonds in the transition state. The isotope effect of the most reactive positions indicates a reaction coordinate mainly of a stretching type, while the least reactive positions have an isotope effect indicating a reaction coordinate intermediate between a pure stretching of the carbon-hydrogen bond concerned and a "rocking" mode involving both carbon-hydrogen bonds.

It is hard to completely exclude a similar single-stage mechanism for the hydrogen exchange, but it seems that an appreciable partial stretching character has to be assumed simultaneously, and a single-stage mechanism including stretching movements of both the incoming and the outgoing substituent can hardly be realized in those substitution reactions which have no observable isotope effect, e.g. nitration, under the same conditions. Thus, if it were attempted to explain the absence of a measurable isotope effect in, for instance, nitration by the assumption of a single reaction step and pure "rocking" reaction coordinate, an essential difference in this respect would have to be admitted.

The vibrational models used in this paper must not be applied to substitutions other than hydrogen exchange, because no other substituents can be regarded as "light" compared to the carbon atom at the centre of reaction. Some remarks, however, could be made in this connection. As pointed out by Streitwieser *et al.*

[9], the fairly low out-of-plane frequency of an aromatic hydrogen could be expected to be increased when the entering substituent makes the hydrogen into a tertiary one. (Such an effect could arise from the fact that the hydrogen becomes angularly "coupled" with the new substituent instead of merely with a p orbital taking part in the aromatic conjugation.) On the other hand, as Streitwieser *et al.* also point out, hyperconjugation probably just cancels this effect, as shown by the absence of a hydrogen isotope effect in several substitution reactions. Provided the model of Section 3 were still applicable for a heavy entering substituent, this absence of an isotope effect could be used to calibrate the constant $k_{\delta v \delta w}$ instead of the procedure on page 207. If it is assumed that Eq. (18) will reproduce a frequency of 700 cm^{-1} (the approximate value of the out-of-plane frequency) with one protium atom and one infinitely heavy "substituent atom", it is possible to find what it will give with one protium and one tritium, or one protium and one deuterium, respectively (we assume $r_v/r_w = p$, the symbols $\bar{\nu}_{s(T)}^+$ and $\bar{\nu}_{s(D)}^+$ as on page 207):

$$\frac{\bar{\nu}_{s(T)}^+}{700} = \left(\frac{1 + \frac{1}{3}}{1 + \frac{1}{\infty}} \right)^{\frac{1}{2}}; \quad \frac{\bar{\nu}_{s(D)}^+}{700} = \left(\frac{1 + \frac{1}{2}}{1 + \frac{1}{\infty}} \right)^{\frac{1}{2}}$$

$$\bar{\nu}_{s(T)}^+ = 810 \text{ cm}^{-1}; \quad \bar{\nu}_{s(D)}^+ = 860 \text{ cm}^{-1}$$

or

$$\bar{\nu}_{s(T)}^+ - \bar{\nu}_{s(D)}^+ = -50 \text{ cm}^{-1}$$

This new value gives a prediction of $k_{\text{ArT}}/k_{\text{ArD}} \approx k_{1(\text{ArT})}/k_{1(\text{ArD})} \approx 0.9$ at 25°C and changes nothing in the previous discussion.

It should finally be remarked that all discussions in the present paper have been founded on the assumption that the curvature of the potential-energy surface along the reaction coordinate is vanishingly small. This implies that we have tacitly assumed the tunnel effect to be negligible. According to recent views [10, 11] this is not necessarily so for reactions involving hydrogen transfer. The present information, however, gives insufficient basis for a discussion of the possible presence of this effect in electrophilic aromatic hydrogen exchange.

SUMMARY

The kinetic isotope effects observed in the hydrogen exchange between aromatic positions and acid media are discussed in terms of two transition-state models, one of non-symmetric type and the other of symmetric type. The models include both bonds between the carbon atom at the centre of reaction and the interchanging hydrogens. The ideas presented in Part V of this series [1] are used in setting up a potential-energy function which makes a simple and approximate vibrational analysis possible. Account is taken of the angular interdependence of the two carbon-hydrogen bonds.

It is concluded that the usual two-stage mechanism with two non-symmetric transition states gives the best fit to the experimental data. The reaction coordinate with the most reactive aromatic positions is probably the stretching of one carbon-hydrogen bond. With aromatic

positions of low reactivity the reaction coordinate is probably intermediate between a pure stretching and a "rocking" mode involving both carbon-hydrogen bonds. Particularly the trend shown by the isotope effect, the effect becoming weaker with decreased reactivity, could be explained by an increasing partial "rocking" character.

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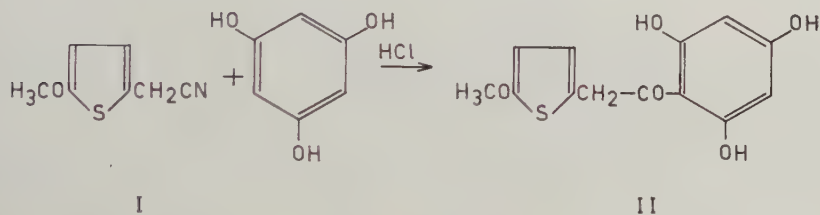
Tryckt den 28 oktober 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

On an anomalous Hoesch synthesis

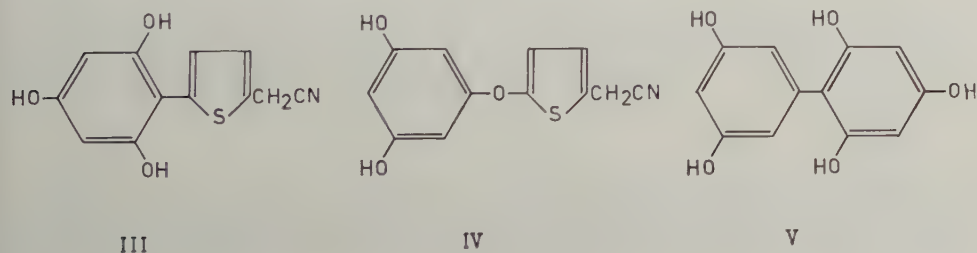
By SALO GRONOWITZ and BO JÄGERSTEN

In connection with work on synthetic analogues to naturally occurring estrogenic isoflavones [1], the present authors reacted 5-methoxy-2-thenyl cyanide (I) with phloroglucinol under the conditions applicable to the Hoesch synthesis (for reviews, cf. refs. [2] and [3]), in order to obtain 2,4,6-trihydroxyphenyl-2-(5-methoxy)-thenyl ketone (II).



After the normal work-up, a crystalline compound of m.p. 232–234°, analyzing correctly for C₁₂H₉NSO₃ was obtained, the IR-spectrum of which (Fig. 1) showed a peak at 4.40 μ (2270 cm⁻¹) characteristic of the C $\equiv$ N group [4]. Evidently, the compound obtained was not the desired ketone.

The elementary analyses and the presence of the cyanide group indicate the probability of the compound formed being either (III) or (IV), hence in order to prove its structure, its NMR-spectrum was investigated.



Its NMR-spectrum in acetone solution (Fig. 2) consists of four different bands (A–D) which have the relative intensities of approximately 3:2:2:2 and is that to be expected for structure (III). Band A consists of two somewhat broadened peaks

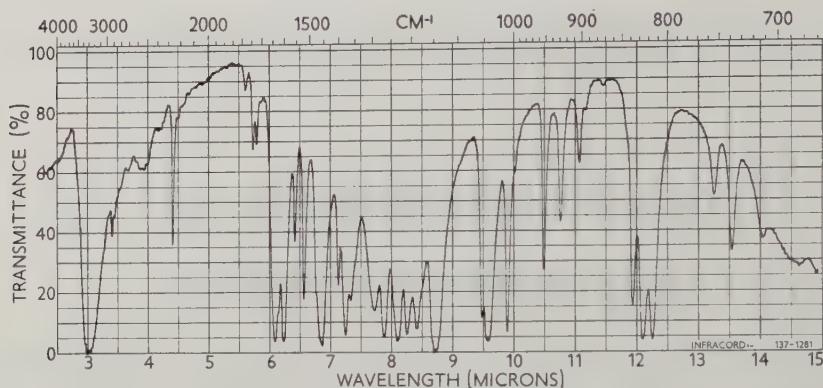


Fig. 1. IR-spectrum of 5-(2',4',6'-trihydroxyphenyl)-2-thenyl cyanide in KBr.

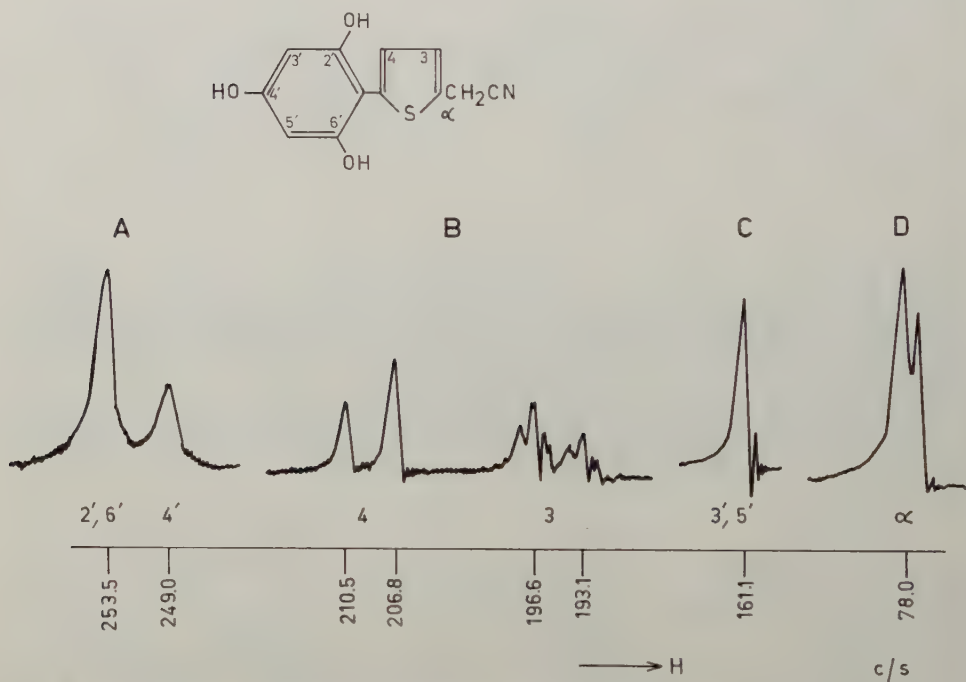


Fig. 2. NMR-spectrum of 5-(2',4',6'-trihydroxyphenyl)-2-thenyl cyanide (30 wt %) in acetone at 40 Mc/s. The figures below the spectrum show the assignments made. The shifts relative to acetone are given in c/s. The recorder gain is 5:5:2:5 for the A, B, C and D parts of the spectrum respectively.

with relative intensities of 2:1 and which have similar shifts as the hydroxyl hydrogens of phloroglucinol dissolved in acetone (-6.13 ppm relative to the solvent peak). In aqueous alkaline solution, these bands disappear and they are therefore ascribed

to the hydroxyl hydrogens. It is interesting to note that the non-equivalence of the hydroxyl group hydrogens in III is apparent in the NMR-spectrum. Band B lies in a region characteristic for thiophene ring hydrogens (ref. [5] and references cited there). The fine-structure of the high field part of this band, which consists of a doublet of triplets, arises from a larger coupling (3.6 c/s) to one hydrogen and a smaller coupling (1.0 c/s) to two equivalent hydrogens. This part of the band is therefore ascribed to hydrogen 3 in the thiophene ring, as it is known that hydrogens in this position couple to alkyl groups in the 2-position [5]. The lower field doublet of band B with a splitting of 3.6 c/s (of the magnitude characteristic of J_{34} in thiophene [5]) is consequently due to hydrogen 4. The sharp peak at 161 c/s (-4.03 ppm) (band C) is ascribed to the two equivalent benzene hydrogens of III and the doublet at 78 c/s (-1.95 ppm) to the CH_2 -hydrogens.

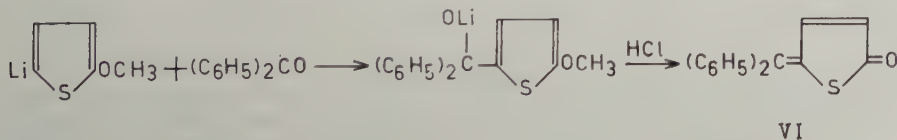
The relative intensities and fine-structure in the spectrum cannot be reconciled with structure IV. Furthermore, the structure of the thiophene band speaks against such a formula, as in the NMR-spectra of 5-methoxy-2-methylthiophene and 5-methoxy-2-thenyl cyanide [5] the order of the 3- and 4-hydrogens is reversed, the resonance of the 4-hydrogen occurring at higher field due to the stronger electron-donating properties of ether groups. The sharp resonance of the benzene hydrogens is also in contrast to what is expected for structure IV.

Upon acetylation, a compound was obtained which analyzed correctly for a triacetyl derivative. It showed no OH-stretching in its IR-spectrum and the peaks ascribed to the hydroxyl hydrogens in the NMR-spectrum of the unacetylated compound were absent. Although the non-equivalence of the CH_3 -hydrogens of the acetoxy groups in the 2' and 6' and the 4'-positions is apparent in the NMR-spectrum in chloroform solution, the resonances of both thiophenic hydrogens coincide in acetone solution.

The evidence presented above proves rather conclusively that the compound obtained is 5-(2',4',6'-trihydroxyphenyl)-2-thenyl cyanide (III).

It is thus obvious that the methoxyl groups of thiophenes differ appreciably in reactivity from those of the corresponding benzene analogues, since *p*-methoxybenzyl cyanide undergoes the Hoesch synthesis in the normal way [6]. The abnormal behaviour in the present case is however not due to the unreactivity of thenyl-substituted cyanides, as Gronowitz and Ekman [1] have found that 2-thenyl cyanide undergoes the Hoesch synthesis, normally giving with phloroglucinol 2,4,6-trihydroxyphenyl 2-thenyl ketone in good yield.

Another case of "anomalous" behaviour of methoxythiophenes has recently been observed by Biggerstaff and Stevens [7], who found that 2-methoxy-5-thienyllithium gave 5-(diphenylmethylidene)-3-thiolene-2-one (VI) upon reaction with benzophenone followed by acid hydrolyses.

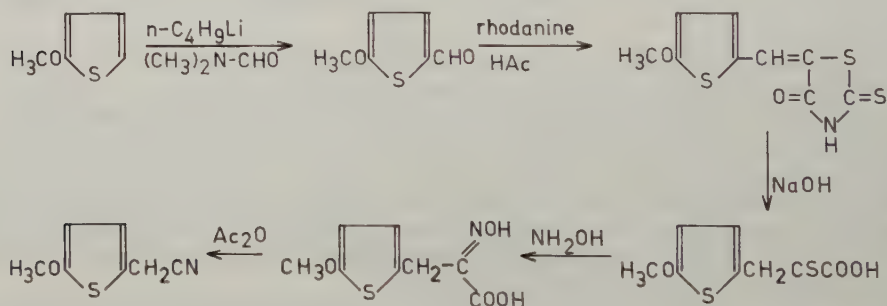


Thus 2-methoxythiophenes differ in many respects from anisoles and their general acid lability was also recognized by Sicé [8].

The condensation reaction observed by us, leading to a hydroxy-phenyl-substituted thiophene, is reminiscent of the autocondensation which phloroglucinol undergoes on heating in the solid state, or better, on refluxing with hydrochloric acid [9]. The structure of the condensation product, phloroglucid, has recently been proved to be 2,4,6,3',5'-pentahydroxybiphenyl (V) [10].

As methoxythiophenes are rather easily available through the cupric oxide [8, 11] or copper [12] catalyzed reactions¹ between iodo- or bromothiophenes and sodium methylate, the present findings might indicate a convenient route to the synthesis of hydroxyphenyl-substituted thiophenes. However, in some preliminary investigations, the condensation of some simpler substituted methoxythiophenes (such as 5-bromo- and 5-methyl-2-methoxythiophene) with phloroglucinol has led to a rather complex mixture of products.

5-Methoxy-2-thiophenealdehyde was obtained in 84 % yield by reacting 5-methoxy-2-thienyllithium (obtained from 2-methoxythiophene [8] and *n*-butyllithium) with *N,N*-dimethyl formamide. Sicé [8] obtained a 67 % yield using phenyllithium in the metalation, whilst Profft [12] obtained a 58 % yield by directly formylating 2-methoxythiophene with *N*-methyl formanilide and phosphorus oxychloride, according to Vilsmeier. The aldehyde was transformed to 5-methoxy-2-thenyl cyanide according to the general method of Julian and Sturgis [13]. Condensation with rhodanine gave (5-methoxy-2-thenal)rhodanine, which was hydrolyzed to 5-methoxy-2-thienylthiopyruvic acid and subsequently converted to the oximino acid with hydroxyl amine. Simultaneous dehydration and decarboxylation with acetic anhydride gave 5-methoxy-2-thenyl cyanide in 31 % yield calculated on the aldehyde.



The more obvious method of using 5-methoxy-2-thenyl chloride for the preparation of the cyanide is impracticable since the chloromethylation of 2-methoxythiophene fails [8].

In a similar way we have prepared 3-methoxy-2-thenyl cyanide in 45 % yield, calculated on the 3-methoxy-2-thiophenealdehyde used, which was obtained from 3-methoxythiophene [11].

Experimental

5-Methoxy-2-thiophenealdehyde

54 g (0.47 moles) of 2-methoxythiophene [8] in 250 ml of dry ether was introduced into a nitrogen-swept four-necked flask equipped with stirrer, reflux-condenser

¹ We found that Sicé's [8] method gave better and more reproducible yields than that of Profft [12] in the preparation of 2-methoxythiophene from 2-iodothiophene.

carrying calcium chloride tube, dropping-funnel and nitrogen-inlet. 470 ml of 1.1 *N* *n*-butyllithium [14] was added dropwise and the reaction mixture refluxed for 30 min. The cooled solution was pressed with nitrogen into a stirred solution of 37 g (0.51 moles) of *N,N*-dimethyl formamide in 100 ml of dry ether. The reaction mixture was allowed to stand over-night and then poured into ice-water. The ethereal layer was extracted with dilute hydrochloric acid, washed with bicarbonate solution and water, dried and fractionated, yielding 57 g (84 %) of 5-methoxy-2-thiophenealdehyde, b.p. 98–100°/3 mm Hg. (Literature values [8, 12], b.p. 79–81°/0.9 mm Hg, b.p. 130–135°/16 mm Hg.)

3-Methoxy-2-thiophenealdehyde

This was prepared as the aldehyde described above, from 68 g (0.59 moles) of 3-methoxythiophene [11] in 400 ml of ether, 440 ml of 1.35 *N* *n*-butyllithium and 65 g (0.89 moles) of *N,N*-dimethyl formamide in 175 ml of dry ether. Upon hydrolyses with water, 30 g of the aldehyde precipitated out and was filtered off. An additional 22 g of aldehyde was obtained from the ether phase. The combined fractions were recrystallized from 60 % aqueous ethanol, yielding 43 g (51 %) of pure 3-methoxy-2-thiophenealdehyde crystallizing in pale yellow needles, m.p. 82–84°.

Anal. $C_6H_6SO_2$ (142.1)

Calc. C 50.69 H 4.26 S 22.55

Found C 50.65 H 4.35 S 22.52

(5-Methoxy-2-thenal)rhodanine

To a solution of 49 g (0.34 moles) of 5-methoxy-2-thiophenealdehyde and 46 g (0.34 moles) of rhodanine in 200 ml of hot acetic acid was added 75 g of anhydrous sodium acetate and the mixture refluxed with stirring for 30 min. The orange-brown product was filtered off with suction, washed with water, small amounts of alcohol and finally with ether. After air-drying, the product (70 g, 79 %) was used in the next step without further purification.

5-Methoxy-2-thienylthiopyruvic acid

70 g (0.27 moles) of the crude (5-methoxy-2-thenal)rhodanine was added to 350 ml of 15 % sodium hydroxide and refluxed with stirring until all was dissolved (30 min). The alkaline solution was cooled thoroughly in an ice-salt mixture and rapidly acidified by adding 350 ml of cold 10 % hydrochloric acid. The product was filtered off with suction and washed with water and a little methanol. After air-drying, the product (60 g) was used in the next step without further purification. A small amount was recrystallized from methanol, yielding an olive-brown crystalline powder, m.p. 191–193°.

5-Methoxy-2-thienylpyruvic acid oxime

To a solution of sodium ethoxide prepared from 18 g of sodium and 580 ml of ethanol was added a saturated aqueous solution of 53 g of hydroxylamine hydrochloride. The precipitated sodium chloride was filtered off, 47 g (0.22 moles) of crude 5-methoxy-2-thienylthiopyruvic acid was added and the mixture refluxed until the evolution of hydrogen sulphide ceased (2 h). The alcohol was then distilled off *in vacuo*, the solid residue dissolved in 120 ml of 5 % sodium hydroxide and the solution filtered from small amounts of sulphur. The oxime acid was then precipitated

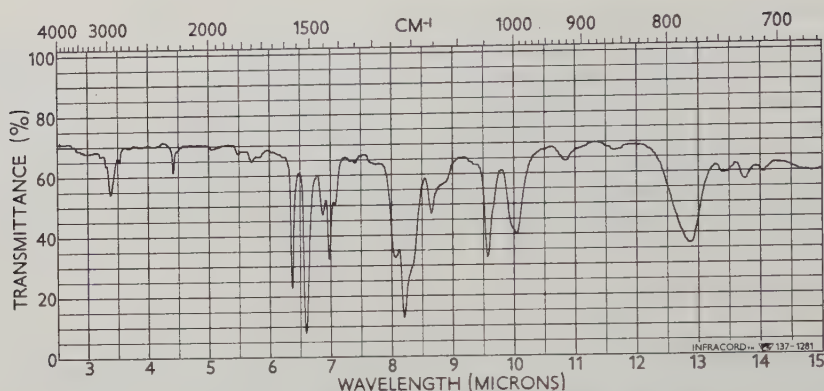


Fig. 3. IR-spectrum of 5-methoxy-2-thenyl cyanide.

by acidifying under cooling with 10 % hydrochloric acid to pH 2. The product was filtered off, washed with a little water and methanol and air-dried. 26 g (55 %) of a light brown crystalline powder, m.p. 143–145° dec. was obtained.

5-Methoxy-2-thenyl cyanide

26 g (0.12 moles) of the dry crude acid oxime and 100 ml of acetic anhydride were introduced into a flask equipped with an internal thermometer, stirrer and efficient reflux-condenser. The mixture was carefully warmed until at 45° a strong exothermic reaction with evolution of carbon dioxide started, and it was necessary to apply a cooling-bath to keep the temperature at 50–60°. After 20 min, the temperature was raised to 100° and the mixture was then allowed to cool to room temperature. Excess acetic anhydride was distilled off *in vacuo* and the residue treated with water and ether. The ether phase was extracted with sodium carbonate solution, dried and fractionated, yielding 12.5 g (68 %) of 5-methoxy-2-thenyl cyanide, b.p. 110–113°/2 mm Hg, $n_D^{20} = 1.5343$ (IR-spectrum, Fig. 3).

Anal. C_7H_7NOS (153.2)

Calc. C 54.88 H 4.61 N 9.14 S 20.93

Found C 55.23 H 4.71 N 9.18 S 20.85

(3-Methoxy-2-thenal)rhodanine

65 g (90 %) of a light brown product was obtained from 40 g (0.28 moles) of 3-methoxy-2-thiophenealdehyde and 39 g (0.29 moles) of rhodanine.

3-Methoxy-2-thienylthiopyruvic acid

From 65 g (0.25 moles) of crude (3-methoxy-2-thenal)rhodanine and 320 ml of 15 % sodium hydroxide was obtained, as described above, 55 g of crude 3-methoxy-2-thienylthiopyruvic acid, m.p. 212–214° dec., after recrystallization from ethanol.

3-Methoxy-2-thienylpyruvic acid oxime

The oximation of 50 g (0.23 moles) of the thiopyruvic acid was carried out as described above, yielding 41 g (82 %) of the acid oxime, m.p. 121–123° dec.

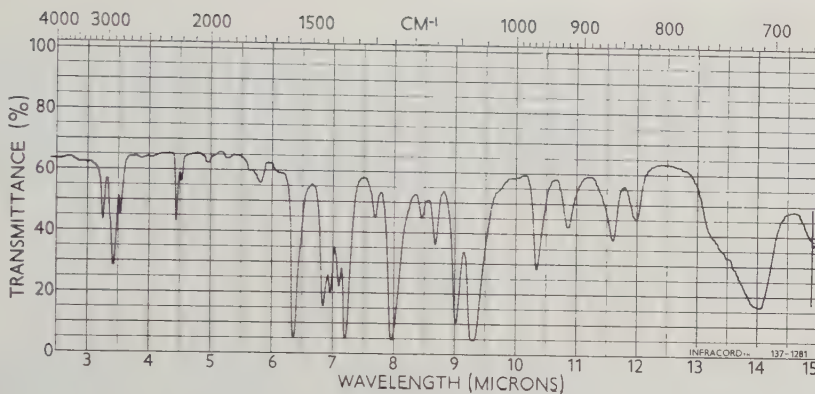


Fig. 4. IR-spectrum of 3-methoxy-2-thenyl cyanide.

3-Methoxy-2-thenyl cyanide

Upon mixing of 40 g (0.19 moles) of the acid oxime and 170 ml of acetic anhydride the reaction started smoothly. The mixture was kept at 30–40° until the evolution of carbon dioxide ceased. The same work-up as described above yielded 17 g (60 %) of pure 3-methoxy-2-thenyl cyanide, b.p. 112–114°/2.2 mm Hg, $n_D^{20} = 1.5386$ (IR-spectrum, Fig. 4).

Anal. C_7H_7NOS (153.2)

Calc. C 54.88 H 4.66 N 9.14 S 20.93

Found C 54.69 H 4.64 N 9.07 S 20.94

5-(2',4',6'-Trihydroxyphenyl)-2-thenyl cyanide

13 g (0.085 moles) of 5-methoxy-2-thenyl cyanide and 10.8 g (0.086 moles) of anhydrous phloroglucinol in 100 ml of dry ether were introduced into a three-necked flask equipped with a gas-inlet tube reaching to the bottom, thermometer and reflux-condenser carrying a calcium chloride tube. A slow stream of dry hydrogen chloride was bubbled in under thorough cooling with an ice-salt mixture. The temperature was kept below 5°. A solid started to precipitate out and when the mixture was saturated with hydrogen chloride, the flask was closed and stored over-night at –15°. The mixture was then again saturated with hydrogen chloride and again stored over-night at –15°. The ether was then decanted off, the residue washed with a little cold ether and then refluxed for 2.5 h with 400 ml of 2 % hydrochloric acid and filtered. Upon cooling, 8 g (38 %) of crystals contaminated with some oily product settled out. Upon recrystallization from 25 % aqueous ethanol, 4.0 g (19 %) of pure 5-(2',4',6'-trihydroxyphenyl)-2-thenyl cyanide crystallizing in colourless prisms, m.p. 232–234°, was obtained (IR-spectrum, Fig. 1; NMR-spectrum, Fig. 2).

Anal. $C_{12}H_9NSO_3$ (247.3)

Calc. C 58.28 H 3.67 N 5.67 S 12.96

Found C 58.18 H 3.71 N 5.61 S 13.11

5-(2',4',6'-Triacetoxyphenyl)-2-thenyl cyanide

1.0 g (0.0040 moles) of 5-(2',4',6'-trihydroxyphenyl)-2-thenyl cyanide was dissolved in 6 ml of dry pyridine, 10 ml of acetic anhydride was added and the mixture allowed to stand for 1 h at room temperature. The mixture was then cooled and 50 ml of ice-cold water was added, which precipitated 1.2 g (80 %) of the triacetate. Recrystallization gave 0.8 g of pure 5-(2',4',6'-triacetoxyphenyl)-2-thenyl cyanide, m.p. 113–116°.

Anal. C₁₈H₁₅NSO₆ (373.3)

Calc. C 57.90 H 4.04 N 3.75 S 8.59

Found C 57.88 H 4.12 N 3.69 S 8.62

In its NMR-spectrum in 30 % acetone solution the resonance of the thiophenic hydrogens occurred at -5.03 ppm, of the benzene hydrogens at -4.99 ppm and of the CH₂-hydrogens (doublet) at -2.12 ppm relative to the solvent. The resonance of the acetyl hydrogens were masked by the solvent. In chloroform solution the resonance of the 2'- and 6'-acetoxy groups occurred at $+5.25$ ppm and of the 4'-acetoxy group at $+5.16$ ppm relative to the solvent.

The IR-spectra were recorded on a Perkin-Elmer Infracord spectrophotometer. The NMR-spectra were obtained with a Varian Associates model V-4300-B high resolution spectrometer operating at 40.00 Mc/s and a flux-stabilized 12 in. magnet from the same company. The magnet sweep was calibrated with the modulation side-band technique using a 50.00 ± 0.05 c/s modulation frequency obtained from the power line. For the large shifts, several side-bands, produced by increasing the modulation field [15], were used and the shifts were measured on the recorder chart by interpolation. The shifts are given relative to the solvents used and are given with the same sign convention as in [5].

SUMMARY

5-Methoxy-2-thenyl cyanide and 3-methoxy-2-thenyl cyanide have been prepared from 5-methoxy-2-thiophenealdehyde and 3-methoxy-2-thiophenealdehyde respectively, by a sequence of reactions involving condensation with rhodanine followed by hydrolyses, oximation, dehydration and decarboxylation.

Condensation of 5-methoxy-2-thenyl cyanide with phloroglucinol by means of hydrogen chloride in ether (Hoesch synthesis) did not lead to the expected thenyl ketone. Instead 5-2',4',6'-trihydroxyphenyl)-2-thenyl cyanide was obtained. Its structure was determined through IR- and NMR-spectroscopy and by preparation of a triacetyl derivative.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Prof. Arne Fredga for his interest in this work and for all facilities put at their disposal. Thanks are also due to Mr. Bo Gestblom for recording the NMR-spectra. The elementary analyses were carried out at the Analytical Department of the Chemical Institute. A grant from *Reservationsanslaget till biträdesjälp m. m. åt docent* to S.G. is gratefully acknowledged.

Chemical Institute, University of Uppsala, May 1961.

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Tryckt den 28 oktober 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

Studies on blood clotting factor VIII

By BIRGER BLOMBÄCK, MARGARETA BLOMBÄCK and INGEMAR STRUWE

Fraction I-0 of human plasma prepared by the glycine method of Blombäck and Blombäck (1956) and containing antihemophilic factor (AHF, f. VIII) has been further purified in our laboratory during the last few years. Thus the antihemophilic factor can be partially separated from the fibrinogen by precipitating an AHF-fibrinogen complex at a low ionic strength in the presence of glycine and at a low ethanol concentration. The precipitate is fraction I-1A (M. Blombäck, 1958). The fibrinogen content of this fraction represents about 80 % of the fibrinogen in fraction I-0.

Fraction I-1A can be adsorbed on tricalcium citrate, whereby the apparent antihemophilic activity, determined with a recalcification system or a two-stage system recently developed in our laboratory, is adsorbed. The tricalcium citrate precipitate can be dissolved in 0.1 *M* EDTA-solution and a precipitate containing antihemophilic activity is spun down by high speed centrifugation. The precipitate is composed of protein and various lipid material. The phosphorus content of the dried precipitate was about 0.8 %. Fig. 1 shows a scheme of the fractionation.

Fig. 1.

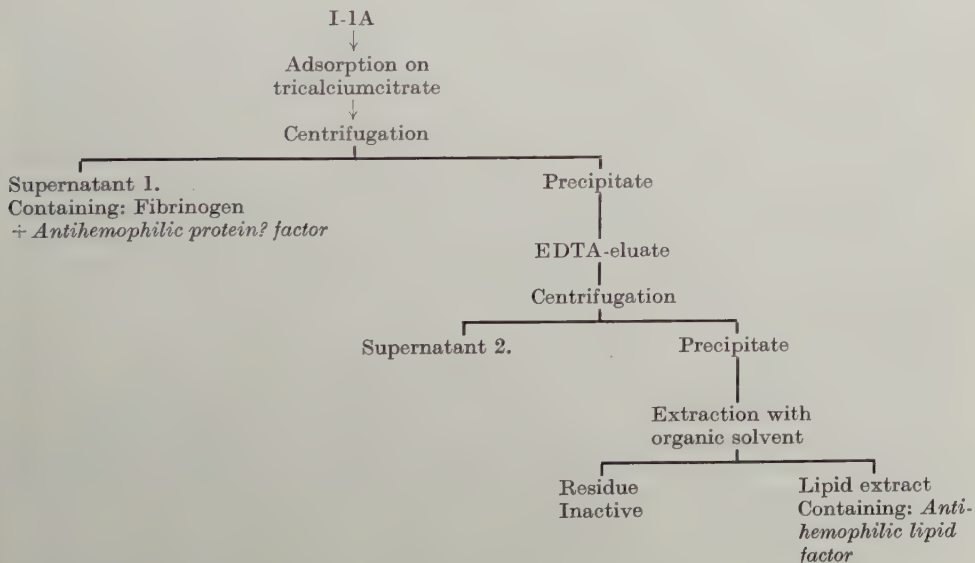


Table 1. Activity of normal and hemophilic plasma fractions before and after addition of lipid extract.

	Activity: NIH Thrombin units/ml/5'		
	Supernatant from normal fraction I-1A	Supernatant from hemophilic fraction I-1A	Lipid extract
No lipid extract	0.18	0	—
With added normal lipid extract	3.49	0.36	0.27

Table 2. Activity of different plasmas before and after addition of lipid extract.

	Activity: NIH Thrombin units/ml/5'			
	Normal plasma	Hemophilia A plasma	Hemophilia B plasma	Normal lipid extract
No lipid extract	0.34	0.06	0.50	—
With added normal lipid extract	4.18	0.21	4.52	0.10

The lipids could be extracted from the precipitate by a variety of organic solvents. Practically all of the phosphorus went into the organic phase. Chromatography of a lipid extract revealed, besides phospho-lipids, glycerides and free fatty acids. Antihemophilic activity was found in the lipid extract, whereas the protein residue after extraction was inactive. If the lipid extracts had no coagulation activity they were found to be devoid of phosphorus. Hitherto no active extract has been obtained from fractions prepared from hemophilia A plasma.

The yield of antihemophilic activity in the lipid extract compared with that in the starting material (I-1A) was however found to be low, usually between 1 and 10 %. It was however found that combination of the lipid extract with the almost inactive supernatant after adsorption on tricalcium citrate (supernatant 1) strongly increased the antihemophilic activity (Table 1). The inactive supernatant left after centrifuging the EDTA-solution (supernatant 2, Fig. 1) could also enhance the activity of the lipid extract. The component of the supernatant necessary for this increase was not found in a corresponding supernatant from hemophilia A plasma (Table 1).

By fractionation of plasma according to Cohn's method 6 the last-mentioned factor is found almost exclusively in fraction I and its sub-fractions.

The antihemophilic activity of various plasmas before and after addition of the lipid extract prepared from normal fraction I-1A is shown in Table 2. As it is shown, addition of lipid extract strongly increases the activity of unaltered normal and hemophilia B human plasma whereas hemophilia A plasma remains practically inactive.

The activity reported has been tested with a new test system composed of

partially purified coagulation factors. The initial speed of thrombin formation from prothrombin is measured in a system composed of antihemophilia A factor (factor VIII), B-factor (factor IX) and factor V among others.

This work has been supported by Research Grant No. H-4295 (R 1) to Professor E. Jorpes from the *National Heart Institute, National Institutes of Health, United States Public Health Service*, and by grants from *Harald och Greta Jeansson's stiftelse* and the *Swedish Medical Research Council*.

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Tryckt den 28 oktober 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

Hydroxyethyl cellulose

A study of its macromolecular properties in solution

By W. BROWN

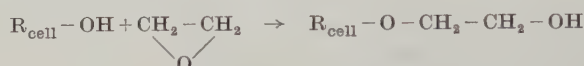
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Introduction

Cellulose is a naturally occurring high polymer consisting of β -D-glucopyranoside units condensed through the 1-4' positions forming an unbranched chain molecule. In the case of native cellulose, the number of anhydroglucose units in a chain may be as high as 7000-10,000. Each anhydroglucose unit in the chain contains a primary hydroxyl group in the 6-position and secondary hydroxyl groups in the 2- and 3-positions. Cellulose is essentially polymolecular, the individual molecules differing widely in the number of anhydroglucose units they contain, the extent of the polymolecularity of a sample having a great influence on its physical properties; it is also a polycrystalline material in which the molecules are thought to be ordered into crystallites (micelles) along parts of their lengths and these separated by amorphous regions. The system may be visualized as a random assembly of crystalline and non-crystalline regions on the sub-microscopic scale. Cellulose has many properties desirable in a high polymer, although it is insoluble in all but a few complex solvents, for example, cupriethylenediamine, *cadoxen* [67], etc. However, it may be readily transformed into useful derivatives such as esters and ethers which are soluble in a wide range of solvents. The water-soluble derivatives of cellulose are increasingly important industrially, but as yet very little is known about their physico-chemical properties in solution. This paper describes the study of a water-soluble, non-ionic ether, hydroxyethyl cellulose, having a broad range of molecular weight.

The etherification of cellulose usually consists of the preparation of alkali cellulose by the interaction of cellulose with dilute sodium hydroxide solution followed by the reaction of the alkali cellulose with the etherifying reagent at an elevated temperature. In such a preparation, the purpose of the sodium hydroxide is to swell the cellulose and to catalyze the reaction (swelling can be considered as a solution of the solvent in the polymer). For a highly substituted product, three or more moles of sodium hydroxide per glucose residue are used in the preparation of the alkali cellulose. The after-treatment varies with the process but normally involves neutralization of the sodium hydroxide, followed by washing with a non-solvent and drying. The degree of polymerization of the product depends upon the nature of the cellulose starting material, but it is known that there is substantial depolymerization of cellulose chains in alkaline media particularly at high temperature. Hydroxyethyl cellulose is generally prepared by reacting alkali cellulose with ethylene oxide in the presence of an inert solvent at an elevated temperature.



This reaction differs from other etherifications of cellulose in that substitution not only takes place on the cellulose hydroxyls but also by addition of ethylene oxide to the primary hydroxyl of the substituent hydroxyethyl group, with the formation of polyethylene oxide side chains. The total substitution will thus be expressed in this paper as *M.S.*, referring to moles of ethylene oxide per anhydroglucose unit.

It is appropriate at this stage to briefly discuss what is known about the solubility of hydroxyethyl cellulose and the stability of the solutions. Solubility of the polymer may be visualized as being due to the opening up of the structure by the bulky substituent groups and probably occurs by preliminary dissolution of the solvent in the polymer. This process takes place more readily at lower temperatures as the solubility behaviour is the reverse of the normal, the solubility increasing with decreasing temperature. The low substituted hydroxyethyl celluloses, *M.S.* < 0.1, can only be dissolved by freezing and thawing a suspension of the material in alkali. Hydroxyethyl cellulose is soluble in aqueous alkali at *M.S.* > 0.1 and becomes increasingly water-soluble as the *M.S.* rises above this figure; above *M.S.* > 1.6, it becomes increasingly soluble in ethanol. These limits are, of course, very approximate, as the solubility also depends on factors other than the extent of substitution.

Various aspects of the solubility of cellulose ethers have been discussed by Jullander [1]. Due to the polycrystalline nature of the cellulose starting material, various parts of the molecular chains are more difficult to etherify than others and one can expect that submicroscopic aggregates, fibres and partially dissolved fibres and swollen fibre residues (gel particles) will remain suspended in the solution. This material may be present in small quantities but can seriously disturb dilute solution measurements. However, some of this material is dissolved on freezing and thawing the solution, accounting for the irreversible increase in viscosity of the solution after this treatment.

Hydroxyethyl cellulose solutions are stable over a wide pH range and are also stable over a broad temperature region. Low concentrations of water-soluble esters and ketones have a precipitating effect, whereas alcohols, carboxylic acids, aldehydes, amines and polyhydroxy compounds are tolerated in much larger quantities. Of the common salts, only sulphates and phosphates precipitate hydroxyethyl cellulose

from solution, presumably by reducing the state of hydration. Other known solvents for water-soluble hydroxyethyl cellulose are dimethyl formamide and diethyl acetamide.

The object of the present study was to make a broad characterization of the hydroxyethyl cellulose molecule in dilute solution by estimation of various molecular parameters depending on the behaviour and configuration of the molecule. Molecules of cellulose derivatives are known to be highly extended and inflexible in solution compared to many polymers whose molecules in solution can be approximated by randomly coiled chains. In addition to studying the behaviour of the hydroxyethyl cellulose molecule in relation to such flexible molecules, it is of interest to compare it to other cellulose derivatives. Differences in the behaviour of different cellulose derivatives are caused to a great extent by the nature of the substituent groups and also the manner of their distribution in the chain. The hydroxyethyl cellulose studied here also has some of the substituent in the form of polyethylene oxide side chains, terminating in the hydrophilic hydroxyethyl group. These bulky substituent groups would be expected to have a marked influence on the configuration and hence the behaviour of the molecule in dilute solution. It may be noted here that Froment [2] has recently described some measurements on a low molecular weight, water-soluble hydroxyethyl cellulose.

The contents of this paper are briefly outlined below.

(a) Most macromolecular substances are heterogeneous with respect to molecular weight and so it is desirable to separate the material into fractions with restricted molecular weight ranges before examining its properties. The fractionation of hydroxyethyl cellulose is described in section 1.

(b) The properties of a cellulose derivative are governed by a complexity of factors, one of which is the distribution of the substituent groups. This problem is discussed in section 2.

(c) Viscosity measurements on dilute polymer solutions are extremely useful for obtaining information regarding the size and shape of the polymer molecules and these measurements are dealt with in section 4.

(d) Sedimentation and diffusion measurements (sections 5 and 6) in combination with the polymer's partial specific volume described in section 7, provide a weight average molecular weight. In addition, the former measurements provide information on the molecular weight distribution of the polymer.

(e) The combined information from viscosity, sedimentation and diffusion measurements is discussed in section 8.

(f) Osmotic pressure measurements, described in section 9, provide the number average molecular weight. Comparison of this value with the weight average molecular weight yields a useful measure of the breadth of the molecular weight distribution of the sample.

(g) Light scattering measurements (section 10) give the weight average molecular weight and the z -average radius of gyration of the polymer molecules. Light scattering measurements also provide information regarding the molecular configuration. Both osmotic and light scattering measurements give values of the second virial coefficient, which is a measure of the polymer-solvent thermodynamic interaction.

(h) In section 11 the behaviour of the hydroxyethyl cellulose molecule is considered in the light of some hydrodynamic theories developed for ideal systems. Information regarding the molecular configuration may be obtained by observing in what respects the molecule deviates from the predicted behaviour of model molecules.

The molecular parameters of hydroxyethyl cellulose are compared to those of other cellulose derivatives such as cellulose trinitrate [41, 44, 45] and particularly to the closely related ethyl hydroxyethyl cellulose [11, 3, 4].

1. Fractionation of hydroxyethyl cellulose

A commercial sample of water-soluble hydroxyethyl cellulose (Cellosize WP 4400¹) was selected, having the molar substitution 1.67, corresponding to 31.2 per cent ethylene oxide. Fractional precipitation from aqueous solution at $25 \pm 0.01^\circ\text{C}$ was used, employing 30 litre and 10 litre Jena glass vessels enclosed to prevent vaporization losses. Acetone was chosen as the precipitant after trial experiments with various water-miscible liquids. The fractionation was carried out in two stages in an attempt to obtain efficient fractionation in the high molecular weight range of the sample. In the first stage, four fractions were produced and in the second, each of these was subdivided into three fractions. Low concentrations were used to improve the efficiency of the procedure; in the first stage, 10 litres of a 3 g/l solution and in the second, 3 litres of an approximately 2 g/l solution. The progress of the precipitation was followed by removing an aliquot of the solution, centrifuging, and evaporating a portion of the supernate to constant weight; this method was sufficiently accurate in spite of "glass" formation on evaporation. When the required quantity of material had precipitated, it was left in contact with the solution with continual stirring for at least 24 hours so as to approach equilibrium. The fractions were removed by centrifugation. A difficulty was experienced in coagulating the final fractions in the second stage, as they remained in suspension even after prolonged centrifugation; coagulation was effected by addition of a small amount of dilute sodium chloride solution. Each fraction was redissolved in water and reprecipitated by addition to a large volume of acetone with vigorous stirring; the material thus obtained had a small particle size, facilitating the subsequent washing and drying. If the precipitated material was dried directly, it formed a hard, horny mass which was difficult to dissolve in water. Each fraction was therefore repeatedly washed with dry acetone by decantation over a period of two weeks in order to remove all water, air dried and, after finally oven drying at 35°C , was obtained in the form of a granular powder, soluble in cold water.

Eleven fractions of about 2 g each were produced and denoted C1 to C11 in order of decreasing molecular weight. The results of the fractionation are given in Table 1, which shows the weights of the fractions, intrinsic viscosities measured in an Ostwald-type viscometer at 20°C and some analyses of the ethylene oxide content expressed as molar substitution. With cellulose derivatives, fractionation may take place according to variations in the extent of substitution as well as molecular weight. However, because of the constancy of the former values for the fractions it is concluded that the fractionation was primarily on a molecular weight basis. The total weight of fractions recovered was 22.6 g from an initial 30 g of polymer. The nature of the material lost was checked by calculating

$$\sum_{i=1}^{i=11} [\eta]_i w_i$$

where $[\eta]_i$ is the intrinsic viscosity and w_i the weight fraction of the i th fraction. The value of 8.4 is in excellent agreement with the intrinsic viscosity of the original

¹ Manufactured by the Union Carbide Chemicals Company, U.S.A.

Table 1. The fractionation of hydroxyethyl cellulose.

Fraction	Weight of fraction g	$[\eta]_{20}$ dl/g	Molar substitution
Original	—	8.35	1.67
C 1	1.93	10.45	
C 2	2.11	11.00	1.62
C 3	0.73	10.45	
C 4	2.10	10.40	
C 5	2.97	10.15	1.63
C 6	2.16	9.70	
C 7	2.08	9.70	
C 8	2.18	9.50	1.64
C 9	1.24	7.75	1.60
C 10	2.63	3.95	1.57
C 11	2.51	1.55	1.68

polymer. This indicates that the loss of high and low molecular weight material during the fractionation has occurred in a corresponding manner.

Fig. 1a shows the integral weight distribution curve for the fractionation obtained by plotting the intrinsic viscosity, $[\eta]_{20}$, against the cumulative weight fraction W . Graphical differentiation of Fig. 1a gave Fig. 1b. It is evident that there is either a substantial uniformity in the molecular weight of the material in the high molecular weight region or, what is more likely, that there is poor fractionation in this range. However, the fractional precipitation of crystalline polymers such as cellulose derivatives is well known to be inefficient. This is because separation of the crystalline material occurs as a poorly reversible process. The rate of crystallization is thus a controlling factor in addition to the equilibrium solubility. Although equilibrium factors would cause the larger molecules to precipitate, their lower crystallization rates may prevent them from doing so. In the present case a non-uniform distribution of hydroxyethyl groups on the molecular chains may also influence the solubility and hence the fractionation.

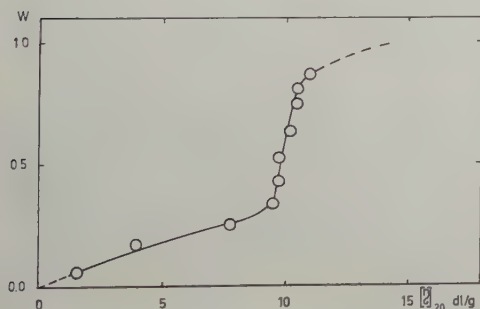


Fig. 1 a.

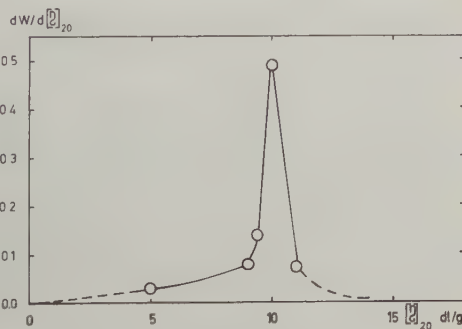


Fig. 1 b.

Fig. 1a. Integral weight distribution curve for the fractionation of hydroxyethyl cellulose. Cumulative weight fraction, W , plotted versus intrinsic viscosity, $[\eta]_{20}$.

Fig. 1b. Differential weight distribution curve obtained by graphical differentiation of Fig. 1a.

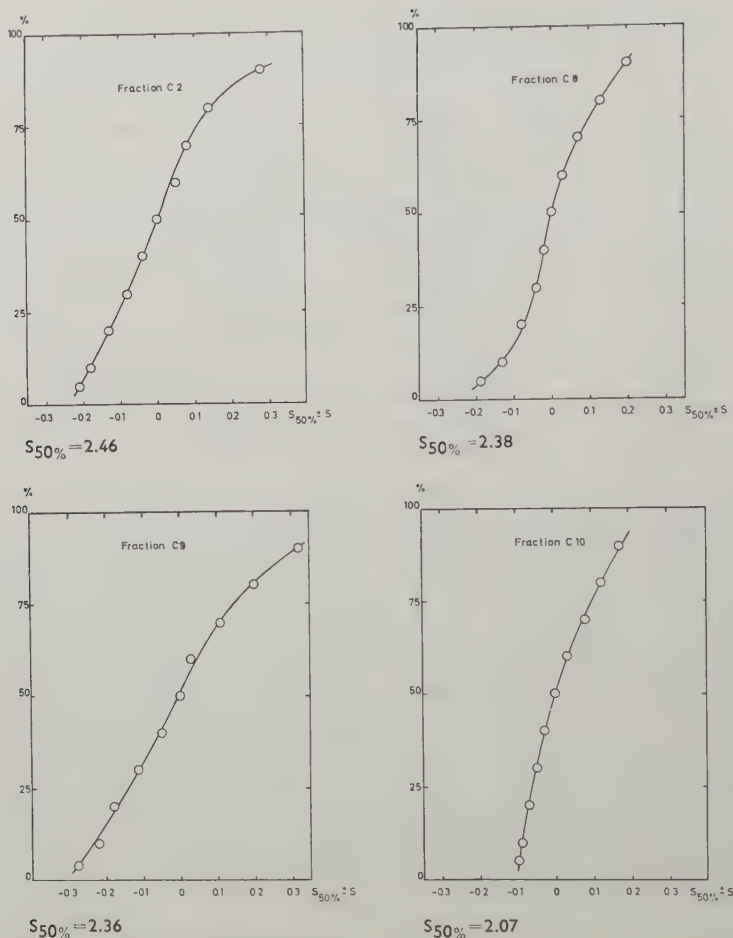


Fig. 2. Integral distributions of sedimentation constants at 25°C for fractions of hydroxyethyl cellulose in water at a concentration of 0.1 g/dl.

Fig. 2 shows integral distribution curves of sedimentation coefficients, s (to be discussed in section 5) obtained from the sedimentation diagrams for four fractions. The relative spread in s for the different fractions gives a comparative measure of their polydispersities. It is also seen from Fig. 2 that, for these fractions, more than 90 per cent of the sample has a rather narrow spread in the sedimentation coefficient and that the "tails" of the distribution have been substantially removed. The molecular weight heterogeneities of these fractions will be further discussed in section 10.

2. The distribution of substituent groups in hydroxyethyl cellulose

The properties of a cellulose derivative will depend not only on the chain length, the polymolecularity and the extent of total substitution, but also upon the distribution of substituent along the cellulosic chain and on the available hydroxyl groups of

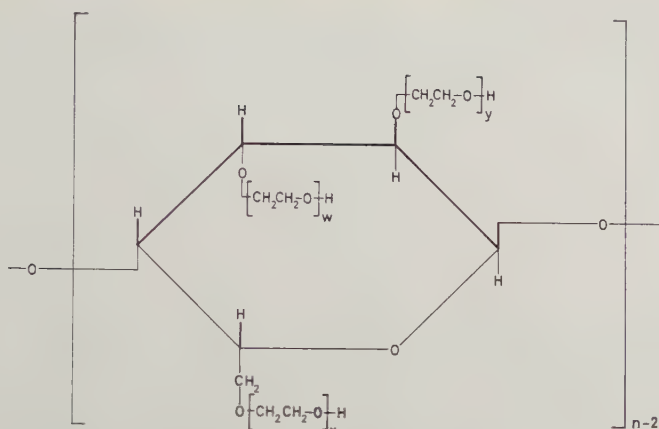


Fig. 3. Schematic diagram of the repeating unit in hydroxyethyl cellulose.

each glucose residue. For example, an even distribution on the chain will confer better solubility than complete substitution in some sections and none in others.

The degree of substitution (*D.S.*) denotes the average extent of total substitution occurring directly on the three hydroxyl groups of each anhydroglucose unit of the chain. Complete substitution therefore corresponds to *D.S.* = 3. With hydroxyethyl cellulose further substitution occurs on the primary hydroxyls of the introduced hydroxyethyl groups leading to polyethylene oxide side chains. It is thus convenient to express the total substitution as moles of ethylene oxide per anhydroglucose unit (*M.S.*). The repeating unit of hydroxyethyl cellulose is shown schematically in Fig. 3.

Partial structure analyses have been made by Morgan [5], Tasker and Purves [6], Cohen and Haas [7], and more complete studies by Brownell and Purves [8], and Croon and Lindberg [9]. The latter authors investigated the distribution of substituents on an hydroxyethyl cellulose of *M.S.* = 0.60 by hydrolyzing the cellulose derivative to monomers (glucose and its ethers) and fractionating these on a carbon column. The relative amounts were in good agreement with values calculated assuming that all glucose units are equally accessible and that the relative velocity constants for etherification of the hydroxyls at C_2 , C_3 and C_6 and the newly introduced hydroxyl on the hydroxyethyl group are 3:1:10:20. The support of the first assumption is important, as the accessibility might be expected to be influenced by the polycrystallinity of the cellulose. Using these values of the relative velocity constants, it is possible to calculate the substitution at the C_2 , C_3 , and C_6 positions of the glucose residue and also the substitution in the form of polyethylene oxide chains.¹ The substitution at the C_2 , C_3 and C_6 positions is given by the expressions [73]

$$S_2 = 1 - e^{-3kt}, \quad (1)$$

$$S_3 = 1 - e^{-kt}, \quad (2)$$

$$S_6 = 1 - e^{-10kt}. \quad (3)$$

and

For polyethylene oxide chain formation

$$dS_{-O-CH_2CH_2-O-} = 20k(S_2 + S_3 + S_6)dt$$

¹ The author is indebted to Professor Bengt Lindberg, Royal Institute of Technology, Stockholm, Sweden, for valuable discussions.

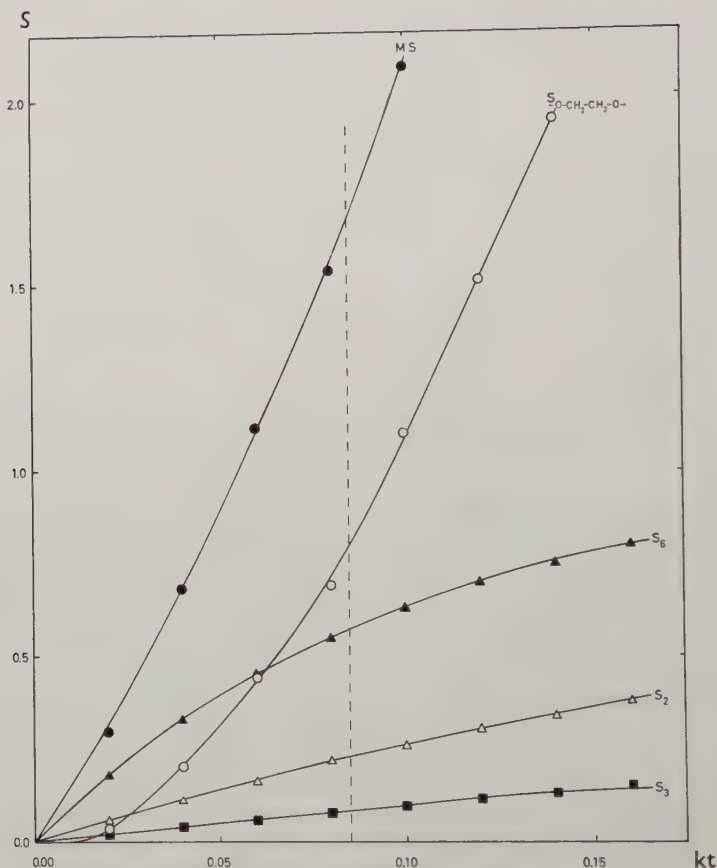


Fig. 4. The total molar substitution, $M.S.$, the substitution as polyethylene oxide chains, $S_{-OCH_2CH_2O-}$, and the substitution at the C_6 , C_2 , and C_3 positions of the glucose residue in hydroxyethyl cellulose. S is the molar substitution, k the rate constant and t the time.

and on integration

$$S_{-O-CH_2CH_2-O-} = 20 \int (1 - e^{-3kt}) dk + 20 \int (1 - e^{-kt}) dk + 20 \int (1 - e^{-10kt}) dk.$$

Evaluating the integration constant by applying the condition $S=0$ at $t=0$ leads to

$$S_{-O-CH_2CH_2-O-} = 60kt - \frac{20}{3}(1 - e^{-3kt}) - 20(1 - e^{-kt}) - 2(1 - e^{-10kt}). \quad (4)$$

Choosing suitable values for kt , one can plot the expressions (1) to (4) as shown in Fig. 4 and summing these curves obtain a curve for the total substitution, $M.S.$ Thus, for a sample of known $M.S.$ one can read off the substitution at the C_2 , C_3 , and C_6 positions, the sum of which is the $D.S.$ and also the substitution in the form of

polyethylene oxide side chains. The sample studied in this paper has a *M.S.* of 1.67 and from Fig. 4 the distribution of substitution is given as follows

$$S_2 = 0.23$$

$$S_3 = 0.08$$

$$S_6 = 0.57$$

$$S_{-\text{O}-\text{CH}_2\text{CH}_2-\text{O}-} = 0.79$$

The *D.S.* is thus 0.88 and 47 per cent of the substituent is in the form of polyethylene oxide chains. Croon and Lindberg [9] calculated *D.S.* = 0.40 for their hydroxyethyl cellulose sample (*M.S.* = 0.60). Employing Fig. 4 one obtains *D.S.* = 0.43, the remainder of the substituent being in the form of polyethylene oxide side chains, which is in excellent agreement. Quinchon [10] has recently described an analytical method using phthalic anhydride, by which the *D.S.* can be determined, and if the *M.S.* is also known, the amount of polyethylene oxide side chains calculated. For an hydroxyethyl cellulose of *M.S.* 1.34 he determined the *D.S.* as 0.63. Using Fig. 4 a *M.S.* of 1.34 corresponds to a *D.S.* of 0.77, which is in reasonable agreement with Quinchon's experimental figure considering the approximate nature of the relative velocity constants.

3. Preparation of solutions

Fractions C2, C8, C9 and C10, approximately covering the molecular weight range of the original unfractionated sample, were selected for the measurements described in this paper. Fresh solutions of these fractions were made for each set of measurements. The solutions were prepared together and treated identically in the following manner. A weighed amount of the fraction was suspended in the required volume of water and shaken for 1 day at 4°C. As the viscosity of a hydroxyethyl cellulose solution depends on the lowest temperature to which it has been submitted [1], the solution was frozen and thawed; this treatment also assists more complete solution in a converse way to freeze drying. Insoluble particles in the solution were removed by centrifugation at high speed (35,000 r.p.m. for 2 hours). Although refractive index measurements before and after centrifugation showed that there was a negligible reduction in concentration, the removal of such material was particularly important for the viscosity and light scattering measurements described in later sections.

For some cellulose derivatives it has been reported (for example, ref. [11]) that extremely low concentrations of salt can affect the viscosity. However, preliminary experiments showed that salt contents of up to 0.1 per cent did not influence the viscosities of solutions of hydroxyethyl cellulose. As the most concentrated polymer solutions used gave no appreciable turbidity with $\text{AgNO}_3/\text{HNO}_3$, the salt level was very much lower than 0.1 per cent. The influence of the surface active nature of the polymer on viscosity measurements has been discussed in some detail by Manley [11]. Hydroxyethyl cellulose in water decreases the surface tension ($\gamma_{25} = 66$ dynes/cm for a concentration of 0.15 g/dl, measured by the capillary rise method). However, a decrease of this magnitude has a negligible effect on the efflux times with the viscometer used in the following measurements.

4. Viscosity

Experimental procedures

The viscometer used was an Ubbelohde-type capillary viscometer, having four bulbs at different heights so that the shear dependence could be determined from measurements at the different velocity gradients. The viscosity η , as determined in such an instrument, may be expressed by a form of Poiseuille's equation

$$\eta = A\varrho t - B\varrho/t, \quad (5)$$

where ϱ is the solvent density, t the efflux time in seconds, and A and B are constants of the instrument; the second term is essentially the kinetic energy correction. By putting equation (5) in the form

$$\eta/\varrho t = A - B/t^2, \quad (6)$$

A and B were evaluated for each bulb as the intercept and negative slope of a linear plot of $\eta/\varrho t$ vs $1/t^2$, using values of η and ϱ for water at the temperatures 0, 15, 25, and 35°C obtained from the International Critical Tables, and the corresponding efflux times of the bulb. The viscometer constants are collected in Table 2.

Viscosity measurements were made on the hydroxyethyl cellulose fractions C2, C8, C9, and C10 at 0, 15, 25, and 35°C in the concentration range 0.02 to 0.1 g/dl. Measurements were made in decreasing order of concentration, dilutions being made by volume in the reservoir bulb of the viscometer and the solution concentrations chosen to give values of the relative viscosity, η_r , in the range 1.1 to 1.9. The relative viscosities were corrected for kinetic energy whenever necessary, using the equation

$$\frac{\varrho_o \eta_s}{\varrho_s \eta_o} = \frac{t_s}{t_o} \left[1 + \frac{B}{A} \left(\frac{1}{t_o^2} - \frac{1}{t_s^2} \right) \right], \quad (7)$$

where the subscripts s and o refer to the solution and solvent respectively.

Table 2. Viscometer constants.

Bulb no. . . .	1	2	3	4
Efflux volume, ml	2.50	2.01	1.21	0.60
Flow time for water at 25°C, sec	53.7	51.3	48.4	52.9
$A \times 10^4$, cm ² sec ⁻²	1.73	1.80	1.86	1.70
$B \times 10^3$, cm ²	21.0	13.5	3.9	1.2
Mean velocity gradient for water at 25°C, sec ⁻¹	1150	970	620	280
Capillary length, cm	29.2			
Capillary radius, cm	0.0325			

*Results and discussion**Shear dependence*

The viscosity is dependent on the shear rate for solutions of long chain molecules, the shear dependence increasing as the molecular weight increases. For each con-

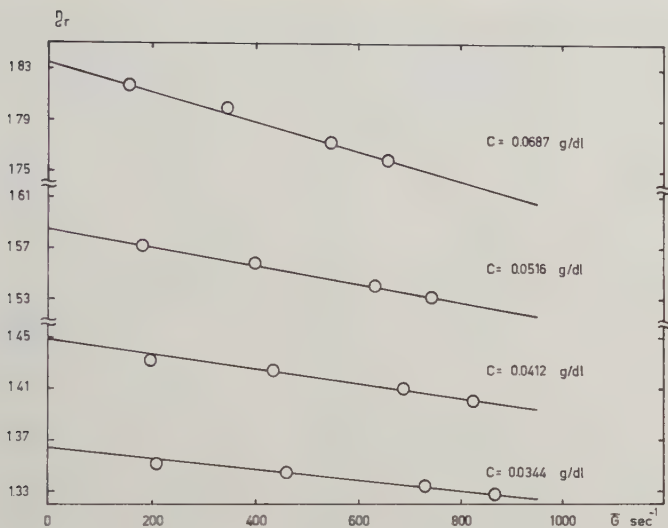


Fig. 5. Dependence of relative viscosity on velocity gradient at different fixed concentrations of hydroxyethyl cellulose fraction C8 at 25°C.

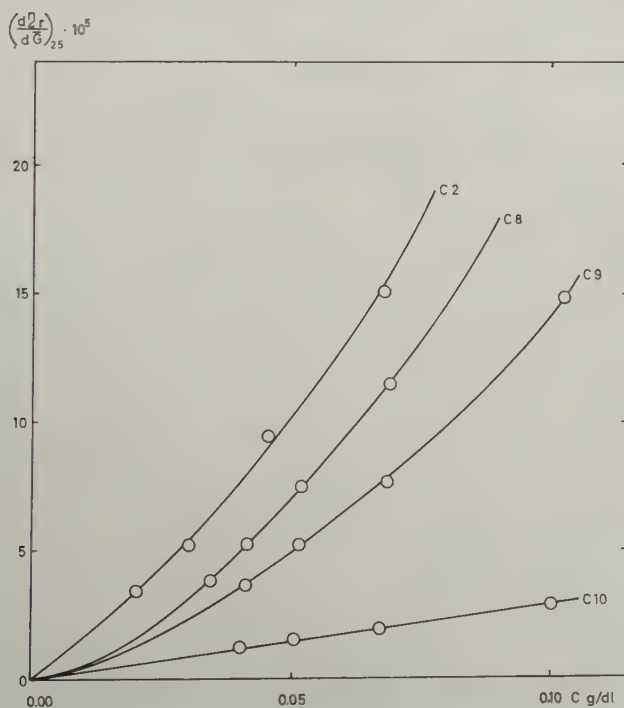


Fig. 6. Gradient dependence of relative viscosity at 25°C as a function of concentration for fractions of hydroxyethyl cellulose in water.

Table 3. Intrinsic viscosities, temperature dependence of intrinsic viscosity and Huggins' constant, k' , for hydroxyethyl cellulose in water at different temperatures.

Fraction	Temperature °C	$[\eta]$ dl/g	$-\Delta[\eta]/\Delta T \times 10^2$ dl/g °C ⁻¹	k'
C 2	0	12.18	6.15	0.87
	15	11.30		0.75
	25	10.62		0.67
	35	10.10		0.57
C 8	0	10.52	5.00	0.80
	15	9.77		0.68
	25	8.95		0.62
	35	8.75		0.49
C 9	0	8.45	3.43	0.74
	15	7.94		0.59
	25	7.54		0.47
	35	7.25		0.40
C 10	0	4.23	1.52	0.57
	15	4.02		0.43
	25	3.81		0.30
	35	3.70		0.22

centration and temperature, the relative viscosity was plotted versus the mean shear rate calculated from the equation

$$\bar{G} = \frac{8V}{3\pi r^3 t}, \quad (8)$$

where V is the volume of liquid delivered in the efflux time t , and r is the radius of the capillary [12]. These plots were linear in the range, $G = 100$ – 1000 sec^{-1} , covered by

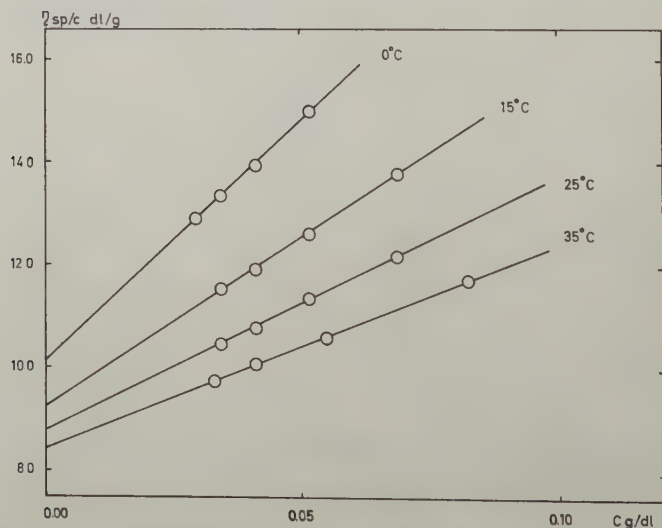


Fig. 7. $(\eta_{sp}/C)_{\bar{G} \rightarrow 0}$ as a function of concentration at different fixed temperatures for hydroxyethyl cellulose fraction C8.

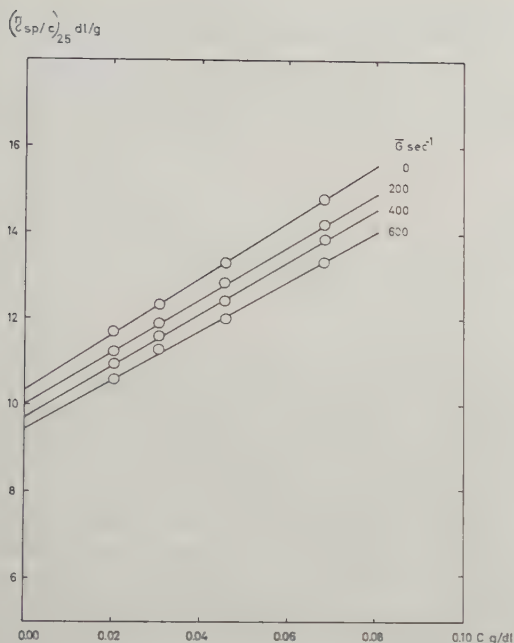


Fig. 8.

Fig. 8. Reduced viscosity at 25°C plotted versus concentration at different fixed rates of shear for hydroxyethyl cellulose fraction C2 in water.

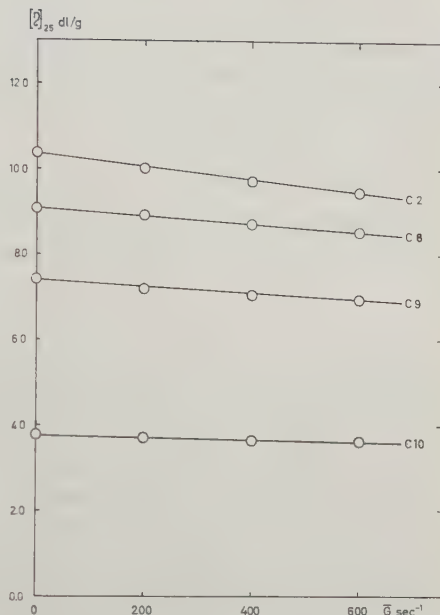


Fig. 9

Fig. 9. Intrinsic viscosity at 25°C plotted versus rate of shear for fractions of hydroxyethyl cellulose in water.

these measurements; an example is shown in Fig. 5 where it is seen that the gradient dependence is small and decreases with decreasing concentration. Pronounced curvature at lower shear rates was considered unlikely. The shear dependence of the relative viscosity, $(d\eta_r/dG)_{25}$, is plotted *vs* concentration in Fig. 6, for the four fractions. The rapid increase in $(d\eta_r/dG)_{25}$ with increasing concentration for the fractions of high molecular weight is probably due to the rapidly increasing molecular interaction. $(d\eta_r/dG)_{25}$ also decreases with decreasing molecular weight, presumably owing to reduced molecular entanglement and facilitated orientation.

The intercepts, at $G = 0$, of the η_r *vs* G plots were used to calculate values of $(\eta_{sp}/C)_{\bar{G}=0}$. $(\eta_{sp}/C)_{\bar{G}=0}$ was plotted against concentration for each fraction at the four temperatures and intrinsic viscosities, $[\eta]$, obtained as the ordinate intercepts of these curves; an example is shown in Fig. 7. Intrinsic viscosities were also determined by plotting $\log (\eta_{sp}/C)_{\bar{G}=0}$ *vs* concentration and excellent agreement was obtained between both procedures for determining $[\eta]$; the values are given in Table 3.

The shear dependence of the intrinsic viscosity was also examined. A plot of $(\eta_{sp}/C)_{25}$ *vs* concentration at different fixed gradients is shown in Fig. 8 for fraction C2 and it is seen that there is a small dependence of $[\eta]_{25}$ on shear rate. From the work of Goldberg and Fuoss [70], it is inferred that the different intercepts reflect changes in orientation or deformation of the molecule with change in shear rate, while

the essentially similar slopes indicate that the molecular interactions are influenced to only a small degree by changes in the rate of shear. The dependence of $[\eta]_{25}$ on G is shown in Fig. 9 for the four fractions and is seen to decrease with decreasing molecular weight. The relationship between $[\eta]_{25}$ and G is approximately linear in the range of G investigated, although curvature might be revealed by measurements over an extended range of shear rate.

The Huggins constant k'

For data obtained at low concentrations ($C < 0.1$ g/dl), (η_{sp}/C) vs concentration curves (as Fig. 7) may be described by the Huggins equation [13]

$$(\eta_{sp}/C) = [\eta] + k'[\eta]^2C. \quad (9)$$

The second term on the right takes account of solute-solvent interaction and k' has been reported to be constant, independent of molecular weight at a given temperature, and to have a value of 0.3 to 0.4 for a linear polymer in a good solvent. However, as shown in Fig. 10 and Table 3, k' increases with increasing intrinsic viscosity at a given temperature, the relationship being linear within experimental error. Timell [32] has found for cellulose trinitrate that k' decreased in a non-linear manner with increasing intrinsic viscosity. Also, as shown in Fig. 11, k' decreases linearly with increasing temperature, the curves for the four fractions having approximately the same slope. This behaviour is markedly different from that of ethyl hydroxyethyl cellulose [11] in a comparable molecular weight region, for which k' shows an irregular variation with molecular weight and remains approximately constant with change in temperature. Many polymers display a higher value of k' in a poor solvent than

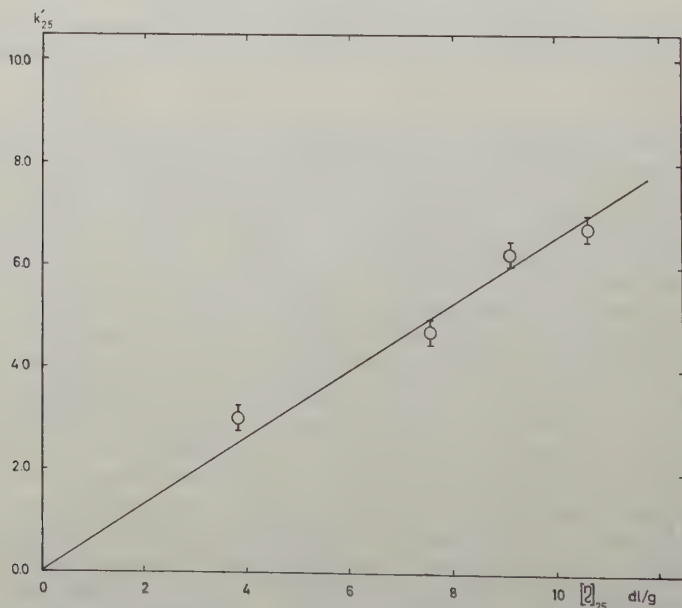


Fig. 10. The variation of the Huggins constant, k' , with intrinsic viscosity at 25°C for fractions of hydroxyethyl cellulose in water.

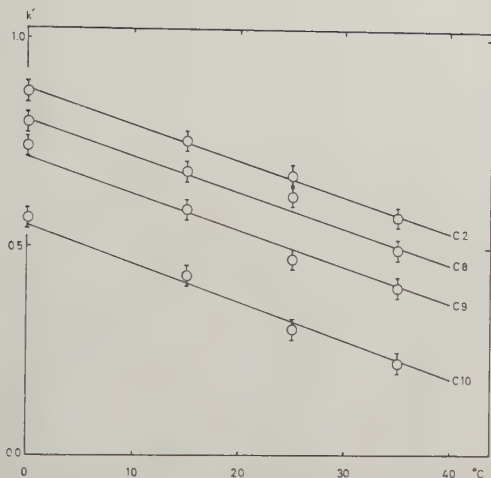


Fig. 11.

Fig. 11. The dependence of the Huggins constant, k' , on temperature for fractions of hydroxyethyl cellulose in water.

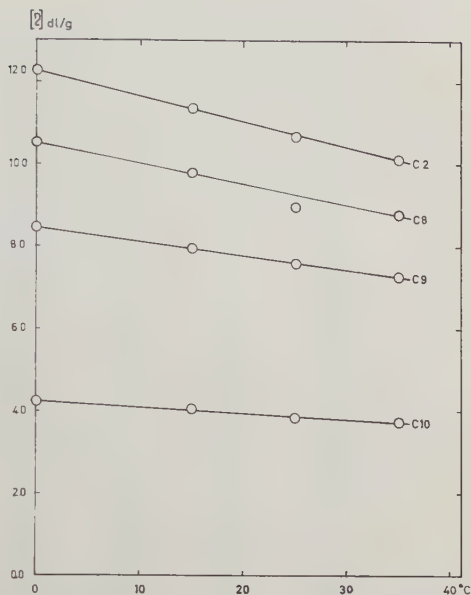


Fig. 12.

Fig. 12. Intrinsic viscosity as a function of temperature for fractions of hydroxyethyl cellulose in water.

in a good solvent. In the case of hydroxyethyl cellulose it is the reverse, k' decreasing as the temperature increases and the solvent becomes progressively poorer. The decrease of k' with increasing temperature may be caused by the nature of the temperature dependence of the thermodynamic interaction of polymer and solvent.

However, the significance of the quantity k' is not yet quantitatively understood. Apart from its variation with solvent power, k' may be influenced by such factors as the dimensions of the molecule in solution, the polydispersity of the sample, molecular branching, and differences in the substituted polymer.

Temperature dependence

Cellulose derivatives are remarkable in possessing a strong negative dependence of intrinsic viscosity on temperature [14, 33]. Hydroxyethyl cellulose displays this behaviour as shown in Fig. 12, the temperature coefficients of intrinsic viscosity being given in Table 3. These values indicate that the solutions of cellulosic chains are particularly sensitive to the solvent environment. Manley [11] suggested that an addition compound is formed with the solvent, which becomes less stable at higher temperatures, while Flory *et al.* [33] have attributed the negative coefficients as due to increasing chain flexibility towards higher temperatures. In Fig. 13, $-\Delta[\eta]/\Delta T$ is plotted versus the degree of polymerization, Z_{ww} , for hydroxyethyl cellulose and ethyl hydroxyethyl cellulose. The temperature coefficients increase approximately linearly with increasing degree of polymerization, this effect being less pronounced for hydroxyethyl cellulose (molecular weight determinations are discussed in later sections).

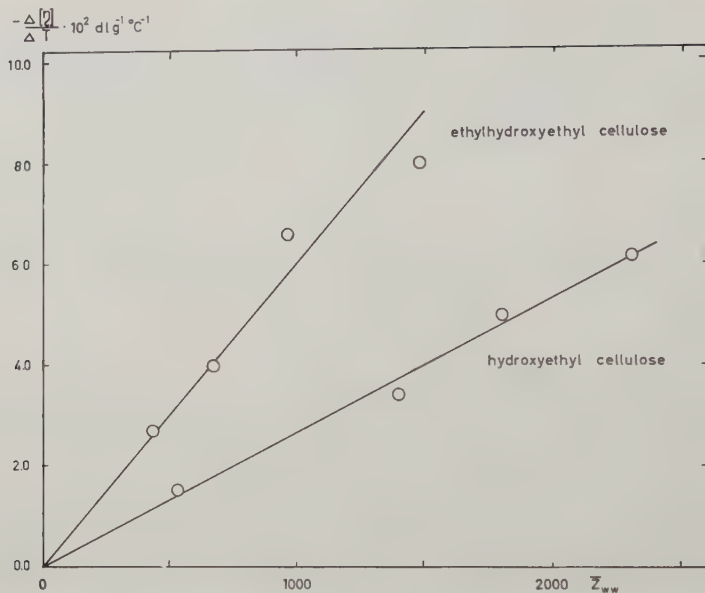


Fig. 13. The negative temperature coefficients of intrinsic viscosity plotted versus degree of polymerization, Z_w , for hydroxyethyl cellulose and ethyl hydroxyethyl cellulose fractions in water.

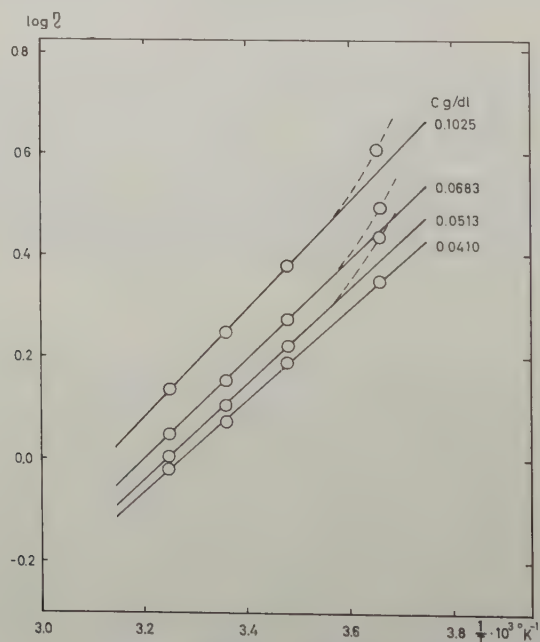


Fig. 14. Logarithm viscosity in millipoises plotted against reciprocal absolute temperature for hydroxyethyl cellulose fraction C9 in water.

Table 4. Viscosities, η , at different temperatures and activation energies of viscous flow, E_A , for hydroxyethyl cellulose fractions in water.

Fraction	C g/dl	η centipoise				E_A kcal/mole
		0°C	15°C	25°C	35°C	
C 2	0.0690	—	2.35	1.75	1.35	4.75
	0.0460	3.07	1.86	1.40	1.11	4.45
	0.0307	2.57	1.56	1.19	0.97	4.13
	0.0205	2.23	1.41	1.09	0.89	3.94
C 8	0.0687	—	2.12	1.59	1.23	4.63
	0.0516	3.03	1.83	1.39	1.09	4.35
	0.0412	2.74	1.66	1.27	1.02	4.24
	0.0344	2.54	1.56	1.20	0.92	4.12
C 9	0.1025	4.05	2.39	1.77	1.37	4.87
	0.0683	3.12	1.88	1.42	1.12	4.45
	0.0513	2.72	1.66	1.27	1.01	4.28
	0.0410	2.50	1.54	1.19	0.96	4.12
C 10	0.1003	2.70	1.66	1.27	1.06	3.85
	0.0669	2.36	1.47	1.14	0.95	3.74
	0.0502	2.20	1.38	1.08	0.88	3.75
	0.0401	2.10	1.33	1.04	0.85	3.73

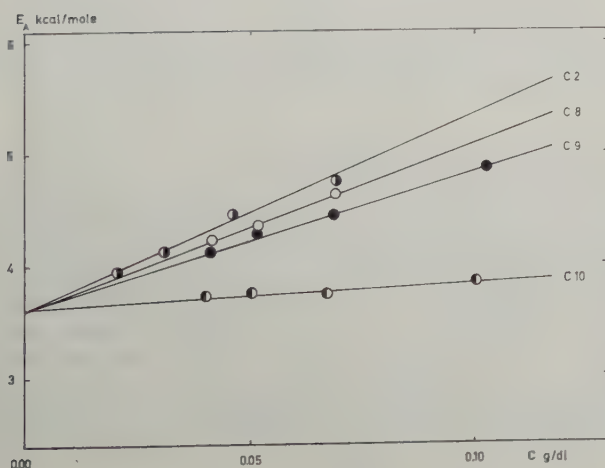


Fig. 15.

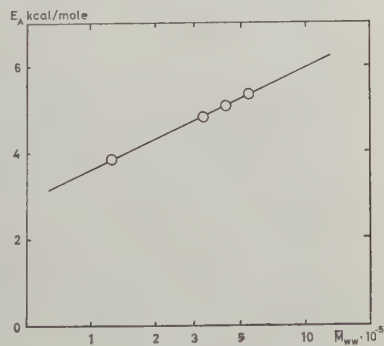


Fig. 16.

Fig. 15. Apparent activation energy of viscous flow plotted against concentration for hydroxyethyl cellulose fractions in water.

Fig. 16. Apparent activation energy of viscous flow plotted against logarithm molecular weight, $\bar{M}_{ww}$, for hydroxyethyl cellulose fractions in water at a concentration of 0.1 g/dl.

The viscosity η may be related to the absolute temperature T by an equation of the form

$$\eta = Q e^{E_A/RT}, \quad (10)$$

where Q is a constant, E_A the apparent activation energy of viscous flow and R the gas constant equal to $1.987 \text{ cal deg}^{-1} \text{ mole}^{-1}$ [14]. Values of η were calculated from equation (5) using the efflux times and values of the viscometer constants A and B for bulb 1 of the four-bulbed viscometer. $\log \eta$ was plotted against $1/T$ at different concentrations for the four hydroxyethyl cellulose fractions; an example is shown in Fig. 14 for fraction C9, the plots deviating from linearity as 0°C is approached. Values of E_A were calculated from the slopes in the linear ranges of these plots. Complete data are presented in Table 4. Fig. 15 shows plots of E_A vs concentration for the four fractions. These are linear and the common intercept gives the activation energy of viscous flow for pure solvent, E_A (water) = 3.6 kcal/mole for the temperature range $273\text{--}308^\circ\text{K}$. This value is in reasonable agreement with published values [15]. In Fig. 16, a plot of E_A vs $\log$ molecular weight is shown, the equation of the line at a concentration of 0.1 g/dl being

$$E_A = 2.38 \log M_{ww} - 8.3. \quad (11)$$

Porter and Johnson [16] have reported a similar relationship between E_A and $\log M$ for polyethylene at 130°C in the molecular weight range of $M_n = 100\text{--}10,000$.

5. Sedimentation

By means of sedimentation measurements, the progress of the polydisperse solute under the influence of the centrifugal field is followed. The sedimentation coefficient may be calculated from the rate of movement of the mean boundary and combination of this quantity with the diffusion coefficient and the partial specific volume of the solute, affords the molecular weight. Distribution curves of sedimentation coefficients provide information on the molecular weight distribution of the samples.

Experimental procedures and results

Sedimentation velocity measurements were made in an oil turbine ultracentrifuge, the general theory and technical details of which are given in a monograph by Svedberg and Pedersen [17]. Recent literature includes a theoretical review by Williams *et al.* [18] and a more general review by Schachman [19]. The sedimentation measurements were carried out at a speed of $60,000 \text{ r.p.m.}$ on hydroxyethyl cellulose fractions C2, C8, C9, and C10 in the concentration range 0.03 to 0.1 g/dl . For each concentration, s was calculated from the initial slope of a $\ln x_t$ vs t curve, where x_t is the position of the boundary at time t . The temperature was normally in the region of 25°C and the sedimentation coefficients were corrected to this temperature using the relationship

$$s_{25} = s_t \cdot \eta_t / \eta_{25}, \quad (12)$$

where η_t and η_{25} are respectively the viscosities of water at $t^\circ\text{C}$ and 25°C . The sedimentation was followed using the Lamm scale method [17]. Complete data are presented in Table 5.

Table 5. Sedimentation data for hydroxyethyl cellulose in water solution at 25°C.

Fraction	Concentration g/dl		$s \times 10^{13}$	$s_0 \times 10^{13}$	K
	C_o	C_{av}			
C 2	0.138	0.131	2.13	5.65	15.8
	0.102	0.088	2.42		
	0.085	0.080	2.45		
	0.071	0.067	2.75		
	0.051	0.048	3.24		
	0.045	0.042	3.32		
	0.030	0.028	3.94		
	0.025	0.024	4.03		
C 8	0.103	0.089	2.37	4.90	11.9
	0.085	0.080	2.42		
	0.075	0.071	2.62		
	0.052	0.048	3.01		
	0.043	0.041	3.33		
	0.041	0.039	3.33		
C 9	0.100	—	2.32	4.45	10.2
	0.093	0.091	2.31		
	0.081	0.078	2.36		
	0.067	0.065	2.57		
	0.041	0.039	3.21		
	0.026	0.023	3.55		
	0.021	0.019	3.80		
C 10	0.150	0.140	1.85	2.82	3.85
	0.100	0.084	2.11		
	0.074	0.070	2.23		
	0.053	0.049	2.36		
	0.050	0.047	2.39		
	0.050	0.042	2.36		
	0.040	0.033	2.51		

The mean position in the boundary, x_t , was taken as that of the maximum point of the curve. This is the usual approximation, although as the curves were not perfectly symmetrical in form, the point corresponding to the position of the second moment of the curve should have been used to locate the position of the mean boundary. However, the magnitude of this approximation is small in the present study, as can be seen by comparing the values of s calculated from the point corresponding to the position of the second moment and from the maximum points of the curves in Table 6.

Table 6. The sedimentation constant at 25°C calculated from the position of the second moment and the maximum point of the boundary, for hydroxyethyl cellulose in water at a concentration of 0.1 g/dl.

Fraction	$s_{\text{second moment}}$	s_{max}
C 2	2.46	2.42
C 8	2.38	2.37
C 9	2.36	2.32
C 10	2.07	2.11

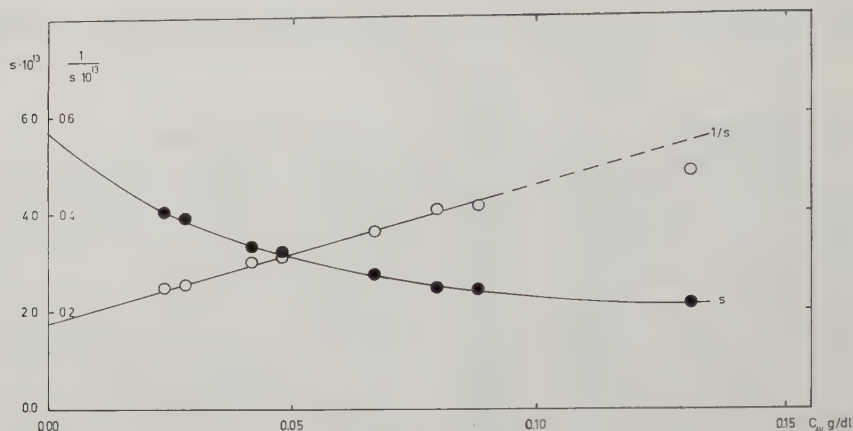


Fig. 17. Extrapolation of sedimentation coefficients to zero concentration for hydroxyethyl cellulose fraction C2.

The contents of the plateau region of the sectorial cell undergo a progressive dilution during the run. The "radial dilution" equation

$$C_t = C_0 \left(\frac{x_0}{x_t} \right)^2 \quad (13)$$

relates the concentration C_t of the solution at time t , to the initial concentration C_0 , in terms of the boundary positions x_t and x_0 at the respective times. The concentrations C_{av} , used when correcting s to its value at infinite dilution s_0 , are those calculated using equation (13) and are given with the initial concentrations in Table 5; the differences between C_0 and C_{av} amount to 5–15 per cent.

For the calculation of molecular weights it is necessary to secure accurate values of s at infinite dilution, where each molecule is considered as moving through the solvent unperturbed by movements of other polymer molecules. However, s becomes increasingly concentration dependent as the concentration approaches zero and simultaneously its measurement becomes more difficult due to factors such as convection in the cell, etc. We must thus depend on values of s at infinite dilution obtained by extrapolation from s -values determined at relatively high concentrations. The magnitude of this extrapolation problem can be appreciated by considering Fig. 17, where s is plotted against C_{av} for fraction C2; there is a rapidly increasing curvature below a concentration of $C_{av} = 0.05$ g/dl. The s -values of fractions C2, C8, and C9 also coincide at concentrations greater than 0.15 g/dl (compare ref. [11]), and only at concentrations of less than 0.1 g/dl do the molecules begin to sediment unhindered and differences in s become significant.

The change in the sedimentation coefficient with concentration arises solely from the concentration dependence of the frictional coefficient f , and s may thus be related to C by a power series of the form

$$s = s_0(1 + KC + K'C^2 + \dots)^{-1}. \quad (14)$$

For the low concentrations ($C < 0.15$ g/dl) used in these measurements, terms above the first in C could be neglected and the plots of $1/s$ vs C were approximately linear for the four fractions. The data were extrapolated in this way to zero concentration and an example is shown in Fig. 17 with the s vs C curve for the same fraction. For all fractions s is approximately independent of concentration above $C = 0.1$ g/dl. The slopes of the $1/s$ vs C plots, K from equation (14), are given in Table 5.

An interesting problem concerns the type of average value corresponding to s yielded by this method of measuring sedimentation constants. Jullander [20] and Kinell [21] have discussed this in detail. In practice, sedimentation velocity experiments using the Lamm scale optical system give a value close to the weight average of s provided the migrating boundary is symmetrical in form. Although this is not, strictly speaking, the case here, it is a sufficiently close approximation, as seen from the comparison of the values in Table 6. s was thus considered as the weight average value for the calculation of molecular weights discussed below.

It is possible to obtain some information regarding the distribution of mass over the different sedimentation coefficients of a polydisperse fraction. The experimental refractive index gradient curve is transformed directly into an integral distribution curve of sedimentation coefficients; this method of boundary analysis was first proposed by Signer and Gross [22]. The spreading of the boundary is a result of diffusion as well as polydispersity and Baldwin and Williams [23] suggested that extrapolation of the distribution to infinite time should give the true distribution. The integral distribution curves for the four fractions at a concentration of 0.1 g/dl, given in Fig. 2, have been corrected in this way. However, two other factors also influence the shape of the boundary. The sharpening effect opposes the boundary spreading and occurs because the concentration on the solvent side is lower than that on the solution side of the boundary. The boundary is also distorted by a series of Johnston-Ogston effects [24] among the polymer species present, resulting in an increase in the concentration of the slower moving species with a corresponding reduction in the amount of the more rapidly sedimenting components. The forms of the curves in Fig. 2 may be influenced by these concentration effects and thus should only be used for comparison between these fractions and not as absolute measures of their polydispersities.

6. Diffusion

Experimental procedures and results

Measurements of free diffusion were made using a precision diffusion apparatus designed and constructed by Claesson and Sahlman in 1953 and which has previously been referred to by Claesson [34]. The spreading of an initially sharp boundary was followed using Rayleigh interference optics [25–27]. This system yields a record of refractive index, in terms of interference fringes, as a function of the vertical coordinate in the cell. The total number of fringes across the boundary, J , is expressed by

$$J = \frac{a \cdot \Delta n}{\lambda}, \quad (15)$$

where a is the cell thickness on the optical path, Δn the difference in refractive index between the solution and solvent, and λ the wavelength of the light source.

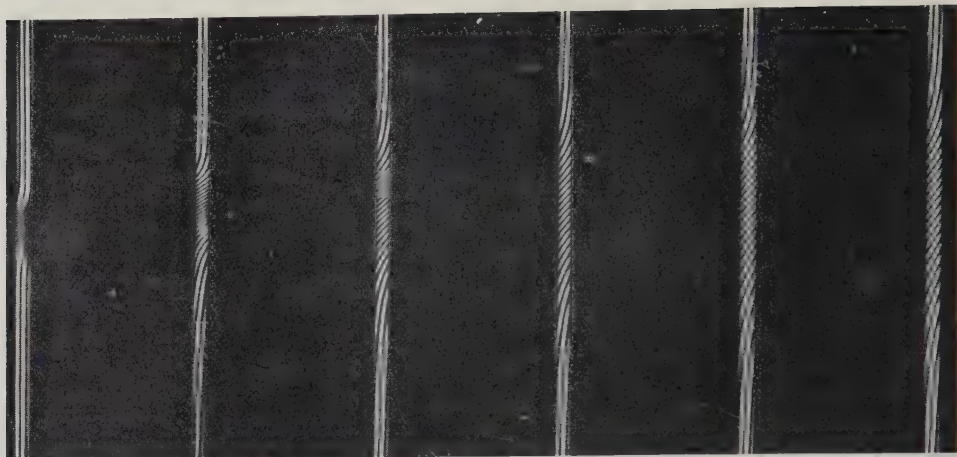


Fig. 18. A recording of a typical diffusion experiment using Rayleigh interference optics. The first exposure on the left was taken two minutes after the formation of the boundary followed by successive exposures at intervals of two hours.

A record of a typical diffusion run is shown in Fig. 18. The boundary has a skewness towards the solvent side indicating that the diffusion coefficient, D , increases with concentration. Values of D may be calculated from such a series of exposures by several methods. In the procedure of Longworth [26] used here, the boundary is assumed to be Gaussian in form and tables of the probability integral can then be used. When the boundary is not perfectly Gaussian, as with these polydisperse fractions having concentration dependent D -values, the Longworth method gives a mean D -value defined by the method of calculation. The probability tables give the normalized "concentration",

$$(2/\pi^{1/2}) \int_0^x \exp(-y^2) dy$$

as a function of normalized "distance",

$$x = -h/(4Dt)^{1/2}. \quad (16)$$

D for a single fringe pair is obtained from the expression

$$D = \frac{(\Delta H/\Delta x)^2}{4tM^2}, \quad (17)$$

where ΔH is the measured distance on the plate between two fringes on opposite sides of the boundary centre, $M = \Delta H/\Delta h$ is the optical magnification factor and t the time in seconds. The distances ΔH could be measured to $\pm 1\mu$. A mean value of D for each exposure was calculated from several fringe pairs.

The diffusion cell was similar to that described by Claesson *et al.* [28]. However, the main body of the present cell was made of metal containing two vertical channels in one of which the boundary was formed, while the other, containing solvent, was

used as a reference path to give the interference fringes. The boundary was formed using the siphoning technique. Difficulties were experienced in forming the boundaries with the highest molecular weight samples. The large viscosity difference between solution and solvent resulted in a pressure difference in the solution and solvent reservoirs and a corresponding disturbance in the boundary. However, by siphoning at a very slow rate and periodically adding fresh solvent with a syringe to equalize the heads, satisfactory boundaries could be produced. The measurements were made in a constant temperature room and care taken to have a steady temperature before forming the boundary. The first exposure was taken immediately after forming the boundary, followed by further exposures at two-hour intervals during the runs which extended over about 30 hours each.

As a check on the experimental arrangement, measurements were made on sucrose solutions. An average value of $D_{25} = 5.26 \times 10^{-6} \text{ cm}^2\text{sec}^{-1}$ was obtained, which compares reasonably with values in the literature (Gosting and Morris $D_{25} = 5.224 \times 10^{-6} \text{ cm}^2\text{sec}^{-1}$ [29]).

At least four concentrations lying in the range 0.2–0.4 g/dl were run on each of the four fractions. Calculations by the Longworth method give a mean value of D for each exposure taken t seconds after formation of the boundary. A plot of Dt against t was made for each run. These plots should be linear and, ideally, intersect the time axis at zero, but in practice these lines meet the time axis on the negative side of the origin, giving the actual starting point of the diffusion. The zero-time correction so determined provides a check on the initial sharpness of the boundary and the linearity of the lines indicates the absence, or otherwise, of disturbance during the run. The zero-time corrections were usually about 2500 seconds, corresponding to a zero-time of about 50 seconds for a sucrose solution diffusing about fifty times faster than the polymer solution. The Dt vs t plots were linear in each case and the mean values of D calculated from the slopes.

Table 7. Diffusion coefficients for water solutions of hydroxyethyl cellulose at 25°C.

Fraction	$\bar{C}$ g/dl	$D \times 10^7 \text{ cm}^2\text{sec}^{-1}$	$D_0 \times 10^7 \text{ cm}^2\text{sec}^{-1}$
C 2	0.179	1.01	0.85
	0.128	0.96	
	0.102	0.94	
	0.075	0.94	
C 8	0.203	1.12	0.96
	0.178	1.11	
	0.152	1.08	
	0.127	1.05	
C 9	0.210	1.25	1.11
	0.184	1.24	
	0.158	1.20	
	0.131	1.21	
	0.105	1.16	
C 10	0.216	1.94	1.86
	0.189	1.94	
	0.151	1.93	
	0.108	1.91	

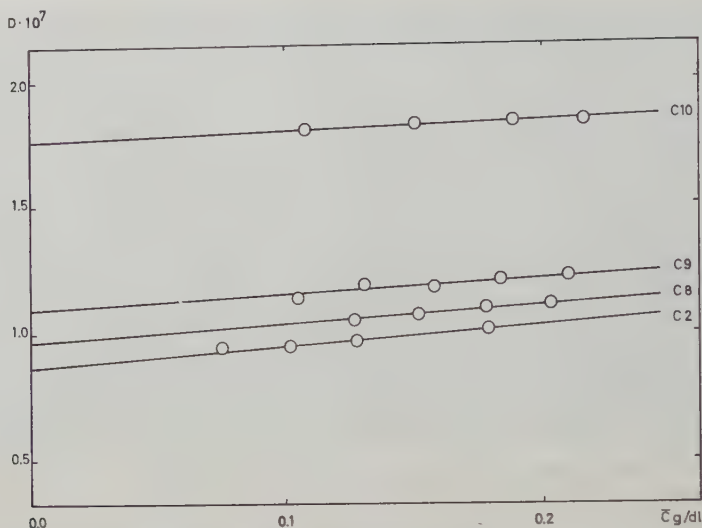


Fig. 19. The dependence of diffusion coefficients upon concentration for fractions of hydroxyethyl cellulose in water.

Measurements of D were made at temperatures of about 23°C and the values corrected to 25°C using the relationship

$$D_{25} = D_t \cdot \frac{\eta_t}{\eta_{25}} \cdot \frac{298}{T}, \quad (18)$$

where η_t/η_{25} is the ratio of the viscosity of water at $t^\circ\text{C}$ to that at 25°C , D_t and D_{25} are the corresponding D -values and T the absolute temperature. Complete data are given in Table 7.

The extrapolations of the diffusion coefficients to obtain the values at zero concentration D_0 , are shown for the four fractions in Fig. 19. The concentration denoted by C is the average value in the diffusion cell. C is defined by $C_0/2$, where C_0 is the original concentration of the solution diffusing against the solvent. The change of D with concentration depends not only on the concentration dependence of the frictional coefficient, f , as in the case of the sedimentation coefficient, but also upon the thermodynamic nonideality. According to Flory [40], it may be expressed as

$$D = kT \frac{[1 + 2\Gamma_2 C + \dots]}{f}, \quad (19)$$

where Γ_2 is related to the second virial coefficient A_2 by $\Gamma_2 = A_2 \bar{M}$ and $\bar{M}$ is the molecular weight. Thus both numerator and denominator terms in equation (19) depend on the concentration. The curves in Fig. 19 are seen to be linear within experimental error and the slopes show a slight decrease with decreasing molecular weight. The dependence of D on concentration is small, as the two sources of concentration dependence tend to nullify each other.

For a polydisperse substance the D -values calculated by the Longworth method correspond to a complicated average value. If the diffusion curve is nearly symmetrical, D should lie between the number and weight averages but closer to the latter. Svensson [30] has developed a method for calculating the height-area coefficient, D_A , from Rayleigh interference patterns; this is a well-defined average again lying between the number and weight averages. However, as the purpose of these measurements was to calculate a quantity characteristic of the weight average molecular weight of each fraction, the Longworth method was chosen.

7. Partial specific volume

Experimental procedures and results

To evaluate the molecular weight it is necessary to know the value of the partial specific volume of the solute, V , in addition to s_0 and D_0 .

Measurements of V were made on three fractions of hydroxyethyl cellulose at 20°C in a concentration range of 0–0.6 per cent by weight. The pycnometer was a single-necked cylindrical glass vessel with a volume of about 25 ml. Filling was by means of a syringe and particular care taken to exclude air bubbles; buoyancy corrections were applied to each weighing. V was calculated from the formula of Kraemer [17]

$$V = V_s \left[1 - \left(\frac{1-w}{m} \right) \cdot \frac{dm}{dw} \right], \quad (20)$$

where V_s is the specific volume of the solution, m is the weight of the pycnometer contents and w is the weight fraction of the solute. (dm/dw) was determined as the slope of a linear m against w plot by the method of least squares. Such a plot is shown in Fig. 20 for fraction C10 and complete results are given in Table 8.

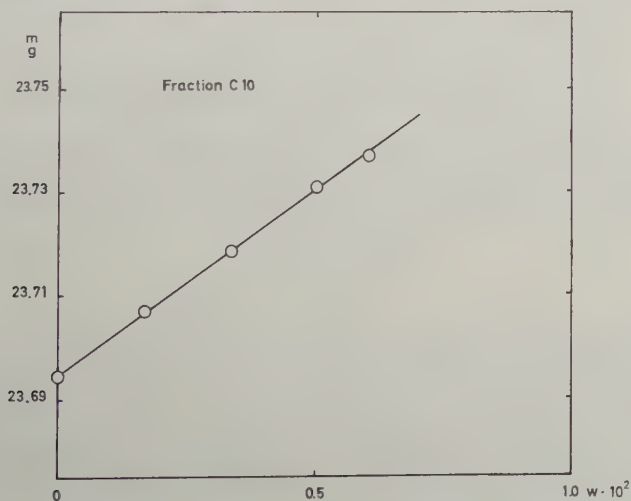


Fig. 20. The weight of the pycnometer contents m , as a function of concentration w for hydroxyethyl cellulose fraction C10.

Table 8. Measurements of the partial specific volume of hydroxyethyl cellulose in water at 20°C.

Fraction	$w \times 10^2$	$\frac{m}{g}$	V_s	V	$(1 - V\varrho)$
C 5	0	23.6945	1.0018	0.704	0.297
	0.1515	23.7061	1.0013	0.705	
	0.3051	23.7166	1.0009	0.705	
	0.4640	23.7270	1.0004	0.705	
	0.5724	23.7353	1.0001	0.705	
C 7	0	23.6945	1.0018	0.694	0.306
	0.1480	23.7037	1.0014	0.695	
	0.2979	23.7152	1.0009	0.695	
	0.4495	23.7270	1.0004	0.695	
	0.5571	23.7347	1.0001	0.695	
C 10	0	23.6945	1.0018	0.701	0.299
	0.1670	23.7069	1.0013	0.701	
	0.3347	23.7186	1.0008	0.701	
	0.5006	23.7309	1.0003	0.702	
	0.6012	23.7368	1.0000	0.702	
Average				0.701	0.301

Andersson [35] has investigated the effect of pressure on V for a number of polymers. For hydroxyethyl cellulose in the range 1–4900 atmospheres he found that V decreased by 1.05 per cent for every 1000 atmospheres increase in pressure. The effect on V of the pressure in the ultracentrifuge rotor at 60,000 r.p.m. (200–300 atmospheres) is therefore negligible. As the partial specific volume is essentially a constant at ordinary temperatures and pressures for a given solute–solvent combination, the average value over the different fractions was used in the calculation of molecular weights.

8. Molecular weights from sedimentation-diffusion

Knowledge of the sedimentation coefficient at infinite dilution, s_0 , the diffusion coefficient at infinite dilution, D_0 , and the partial specific volume, V , of the polymer fraction permits the evaluation of the molecular weight from the Svedberg equation

$$\bar{M} = \frac{RTs_0}{D_0(1 - V\varrho)}, \quad (21)$$

where R is the gas constant equal to 8.314×10^7 ergdeg $^{-1}$ mole $^{-1}$, T the absolute temperature, and ϱ the solution density. Using the values of s_0 and D_0 from Tables 5 and 7, and the average value of V from Table 8, the molecular weights of the fractions were calculated from equation (21) and are given with the corresponding degrees of polymerization in Table 9. For the calculation of the latter quantity, the monomer molecular weight of the hydroxyethyl cellulose, $M.S. = 1.67$, is 236. Various average molecular weights may be obtained from equation (21), depending on the methods used for selecting mean positions in the sedimentation and diffusion

Table 9. Molecular constants of hydroxyethyl cellulose from sedimentation-diffusion data.

Fraction	$\bar{M}_{ww} \times 10^{-3}$	$\bar{Z}_{ww}$	f/f_0	l/d
C 2	545	2310	5.29	172
C 8	420	1780	5.12	161
C 9	330	1400	4.78	140
C 10	125	530	3.96	94

boundaries. Here, s_0 and D_0 have been assumed to approximate to weightaverage values and the molecular weights therefore are denoted $\bar{M}_{ww}$. For a given fraction the difference between $\bar{M}_{wv}$ and $\bar{M}_w$ depends on its heterogeneity; this is discussed more fully in section 10. The most serious limitation to the sedimentation-diffusion procedure for determining molecular weights in the present case is the inaccuracy introduced by the difficult extrapolation of s , and to a lesser extent D , to zero concentration, in addition to the troublesome nature of these measurements on this polymer-solvent system.

The relationship between the intrinsic viscosity and molecular weight can be expressed by the modified Staudinger equation

$$[\eta] = K\bar{M}^a, \quad (22)$$

where K and a are constants independent of molecular weight for a specific solute-solvent system. Once such relationship is established, molecular weights can be obtained directly from viscosity measurements. The exponent a provides information on the flexibility of the molecule in solution, its magnitude depending on the model approximated by the randomly coiled polymer chain; $a \approx 0.5$ for a tightly coiled,

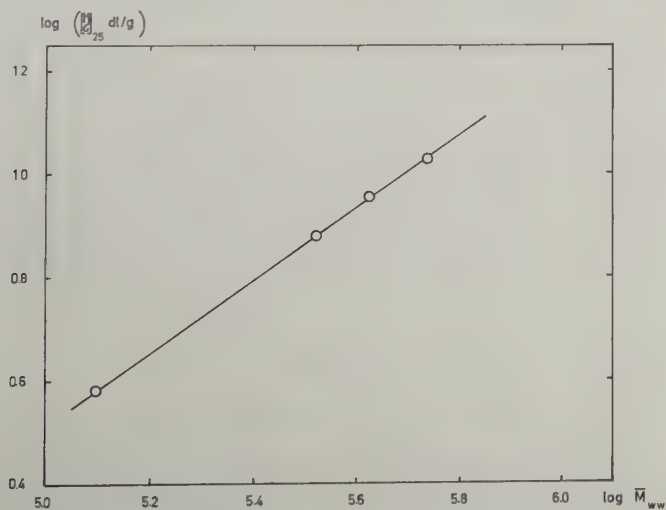


Fig. 21. Logarithm intrinsic viscosity versus logarithm weight-average molecular weight for hydroxyethyl cellulose fractions in water.

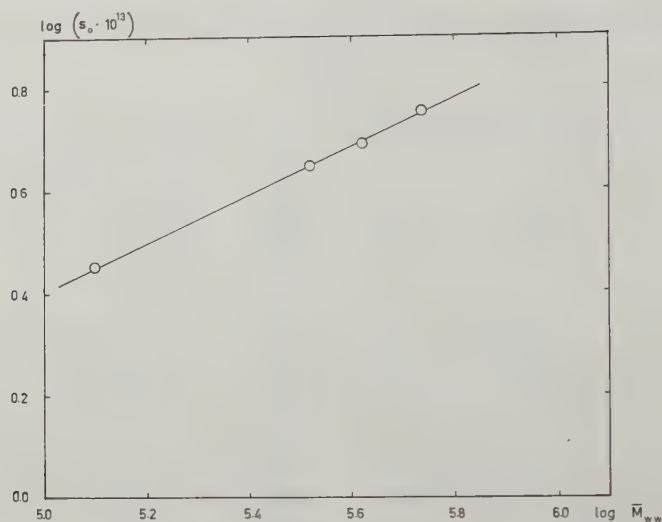


Fig. 22. Logarithm sedimentation coefficient at infinite dilution versus logarithm weight-weight average molecular weight for hydroxyethyl cellulose fractions in water.

impermeable molecule allowing no draining of solvent and $a \approx 0.8$ for a flexibly-coiled molecule allowing free drainage of solvent. Some cellulose derivatives having stiff, extended molecules, for example, cellulose trinitrate, have a value of a close to unity.

Fig. 21 shows a plot of $\log [\eta]_{25}$ vs $\log M_{ww}$; the line may be represented by

$$[\eta]_{25} = 1.03 \times 10^{-3} M_{ww}^{0.70}. \quad (23)$$

The significantly lower value of a for hydroxyethyl cellulose compared to ethyl

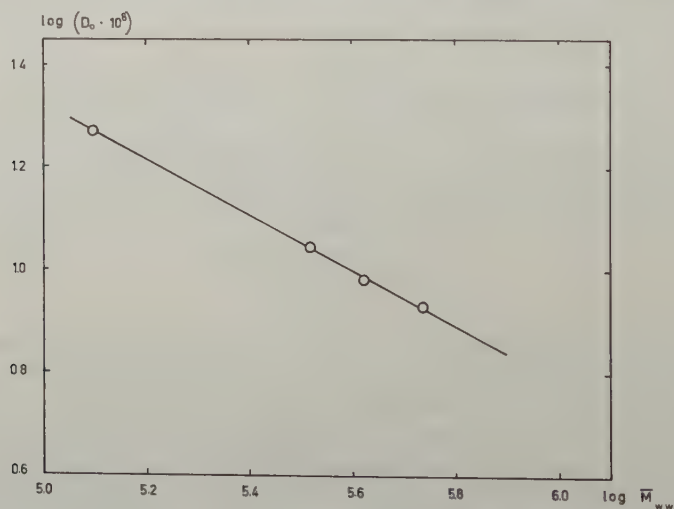


Fig. 23. Logarithm diffusion coefficient at infinite dilution versus logarithm weight-weight average molecular weight for hydroxyethyl cellulose fractions in water.

hydroxyethyl cellulose [11] ($\alpha = 0.8$) indicates a lower extension of the former molecules in solution, possibly as a result of different steric factors.

Fig. 22 shows the dependence of the logarithms of the sedimentation coefficients on logarithm molecular weight, the line corresponding to the relation

$$s_{25} = 1.0 \times 10^{-15} \bar{M}_{ww}^{0.46}. \quad (24)$$

Fig. 23 shows the dependence of the logarithms of the diffusion coefficients on logarithm molecular weight, the equation of the line may be expressed as

$$D_{25} = 8.2 \times 10^{-5} \bar{M}_{ww}^{-0.54}. \quad (25)$$

According to Flory [40], for randomly coiling polymers the exponent in equation (24) should lie between 0.4 and 0.5 and the exponent in equation (25) between -0.5 and -0.6 where the models corresponding to these limits are as given for the exponent in equation (22). The exponents of $\bar{M}_{ww}$ in equations (23), (24), and (25) suggest that the hydroxyethyl cellulose molecule in water solution has a form lying between that of a compact solvent-impermeable coil and a loosely coiled molecule allowing free draining of solvent.

Molecular shape

The frictional ratio f/f_0 , is given by the equation [17]

$$f/f_0 = \left(\frac{1 - V_0}{D_{25}^2 s_{25} V} \right)^{1/3} \frac{\eta_{20}}{\eta_{25}} \cdot 10^{-8}, \quad (26)$$

where f is the frictional coefficient for the molecule, and f_0 the frictional coefficient for the corresponding unhydrated spherical particle; the other terms are as previously defined. The frictional ratio f/f_0 thus equals unity for spherical, unhydrated molecules and is greater than unity when the molecules are asymmetrical or hydrated, or a combination of both. If the molecules are assumed unhydrated and to have an ellipsoidal shape in solution, f/f_0 can be expressed as the ratio of length to diameter l/d of such an ellipsoid. Tables of these ratios have been given by Gralén [31] and values of f/f_0 and l/d are given in Table 9. From the ratio l/d it is possible to obtain information on the molecular dimensions. However, this knowledge is afforded by light scattering measurements (section 10) in a more direct and reliable way. Considerable information regarding molecular dimensions may also be obtained by applying recent hydrodynamic theories of macromolecules to the viscosity and sedimentation-diffusion data (section 11). In comparison with these two latter approaches, dimensional data obtained from the ratio l/d is of limited value.

9. Osmosis

Experimental procedures and results

Osmotic measurements were made in a Fuoss-Mead block-type osmometer provided with an organic liquid manometer; the instrument is shown in Fig. 24. The design is essentially that described by Vink [64], and has later been modified by Palm [66]. The construction is such that the solvent and solution-organic liquid

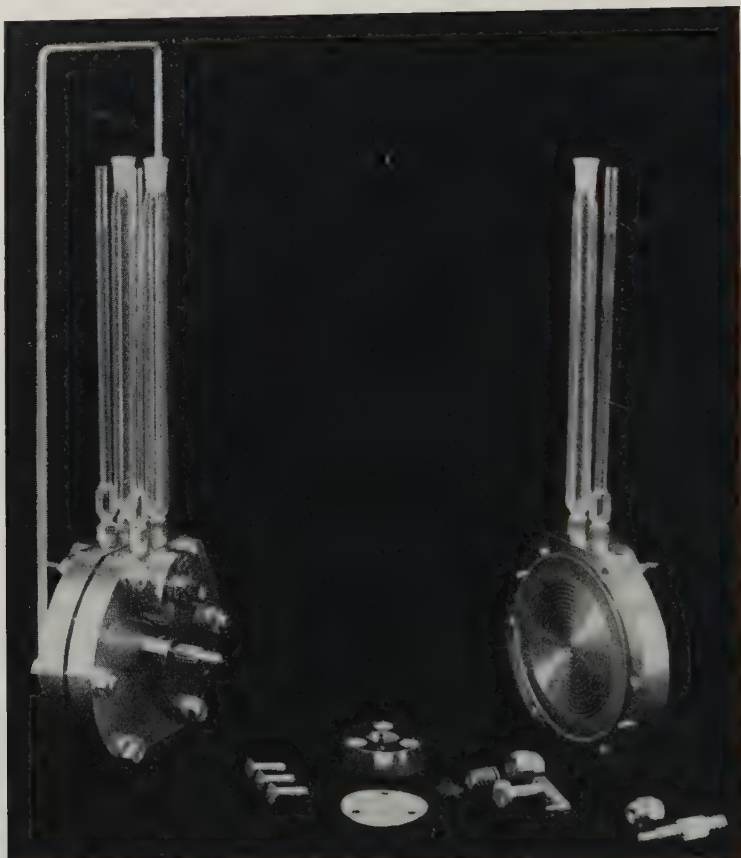


Fig. 24. Photograph of the osmometer. On the left is the assembled instrument and on the right one of the symmetrical halves. A disassembled three-way valve and needle valve are shown in the foreground (ref. 66).

boundaries are located in wide tubes beneath the capillaries, while the air - organic liquid interfaces are situated in the capillaries. This feature facilitates measurements when the solvent or solution is unstable in contact with the atmosphere and also minimizes errors due to surface tension, etc. For the measurements described here, *n*-hexane (b.p. 69°C, ρ_{25} 0.655) was chosen as the manometer liquid.

Commercial viscose membranes were used in preliminary measurements, but it was found in all cases that a finite pressure was developed when solvent was in both chambers of the osmometer and was presumably due to an asymmetrical membrane structure. These membranes were also unsuitably "tight" in view of the high molecular weights of the samples as the half-time to approach equilibrium was about 80 minutes. The membranes finally selected for measurements were *Ultrafeinfilter* "fein" from Membranfilter Gesellschaft, Göttingen. These membranes were initially conditioned in water for a period of 1 to 2 weeks and then further conditioned for 2 to 3 days in the osmometer by frequently flushing the chambers with fresh solvent. Conditioning was judged complete if the excess pressure of manometer liquid on one

side, Δh , did not exceed 0.01 cm. The performance of the osmometer containing solvent in both chambers was checked as follows. The capillary menisci were initially set at different levels and the difference in height, Δh , recorded as a function of time. In all cases, $\log (\Delta h)$ was found to decrease linearly with time. From these plots the half-time to approach equilibrium was found to be about 2.5 minutes.

Osmotic pressure measurements were made on hydroxyethyl cellulose fractions C2, C8, C9, and C10 using the static equilibrium method. The osmotic pressure, π , was measured on the most dilute solution first and then on the other solutions of the fraction in increasing order of concentration. For each concentration, the solution chamber was flushed at least three times with solution before measurement. Adsorption of solute on the membrane was usually less than 5 per cent and equilibration of the membrane with solution was attained by standing overnight. The osmometer was then flushed again with solution and π measured from both sides of the final equilibrium position. This procedure was facilitated by the short "half-time" of the membranes. After the determination of π for a particular concentration, the solution chamber was flushed with the next concentration of the fraction as described above.

All measurements were carried out at $25 \pm 0.01^\circ\text{C}$. Using a cathetometer reading to 0.001 cm, readings were made on the four menisci; the air - organic liquid interfaces, a_1, b_1 , in the capillaries and the solvent and solution - organic liquid interfaces, a_2, b_2 , in the wide tubes, where a and b refer to the two symmetrical sides of the osmometer. The excess pressure on the a side is then given by

$$\pi = (a_1 - b_1) + \frac{\varrho - \varrho_0}{\varrho_0} (a_2 - b_2), \quad (27)$$

where ϱ and ϱ_0 are respectively the densities of solution and manometer liquid.

Table 10. Osmotic data for hydroxyethyl cellulose in water at 25°C .

Fraction	C g/dl	Δh mm water	$(\pi/C) \times 10^3$ atm dl/g	$\bar{M}_n \times 10^{-3}$	$A_2 \times 10^4$ ml mole/g ²
C 2	0.152	1.63	1.04	380	11.5
	0.169	1.95	1.12		
	0.254	3.48	1.33		
	0.305	4.82	1.53		
	0.338	5.57	1.60		
C 8	0.223	2.76	1.35	310	11.0
	0.251	3.88	1.50		
	0.355	5.87	1.70		
	0.396	7.51	1.84		
C 9	0.100	1.45	1.58	185	11.3
	0.224	3.95	1.92		
	0.300	6.82	2.13		
C 10	0.156	3.84	2.39	125	11.6
	0.161	4.02	2.42		
	0.243	6.65	2.65		
	0.312	9.15	2.84		
	0.323	9.60	2.88		

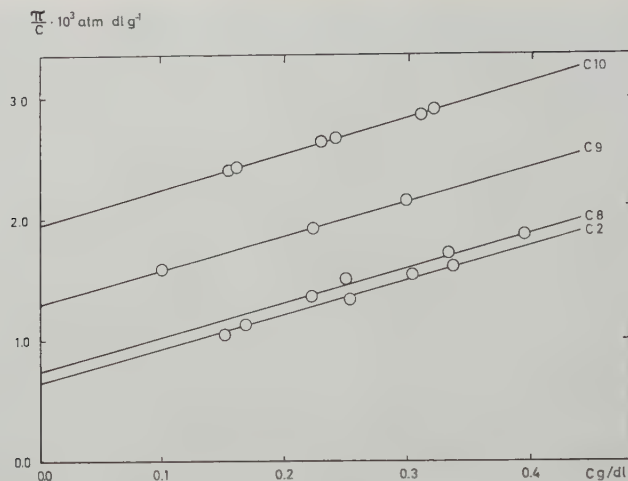


Fig. 25. Reduced osmotic pressure, (π/C) , as a function of concentration for hydroxyethyl cellulose fractions in water at 25°C.

Measurements were made in the concentration range 0.1 to 0.4 g/dl and at these concentrations the measured osmotic pressures of the high molecular weight fractions C2 to C9 were small, as seen in Table 10. However, it was necessary to employ this concentration range owing to the probability of a non-linear variation of the reduced osmotic pressure with concentration at higher concentrations and the difficulty of efficiently flushing the osmometer channels with the highly viscous solutions. The concentration of the polymer solution at the time of filling the instrument was taken as the true concentration at the time of measuring π . The error thus introduced should be negligible owing to the extremely small transference of solvent across the membrane. Solute permeation through the membrane was of no significance, in spite of its high permeability to solvent, as the equilibrium position was constant for several days.

The reduced osmotic pressure may be expressed as a function of concentration by a power series of the form

$$(\pi/C) = (\pi/C)_0 [1 + \Gamma_2 C + \dots], \quad (28)$$

where $(\pi/C)_0 = RT/\bar{M}_n$ represents the limiting value of (π/C) at infinite dilution and Γ_2 is a parameter which depends on polymer-solvent interactions and is related to the second virial coefficient, A_2 , by

$$\Gamma_2/\bar{M}_n = A_2. \quad (29)$$

Equation (28) should hold for concentrations for which $(\pi/C) < 3(\pi/C)_0$. It may be noted that Tompa [65] has comprehensively discussed the existing theories dealing with the extrapolation of osmotic measurements to infinite dilution.

In Fig. 25 (π/C) is plotted *vs* C for the hydroxyethyl cellulose fractions, where π is expressed in atmospheres and C in g/dl. Values of $\bar{M}_n$, obtained from the intercepts according to equation (28), are listed in Table 10. The second virial coefficients were

Table 11. Determination of the θ -point for hydroxyethyl cellulose in water; osmotic measurements on fraction C 10 at 35.8° and 6.9°C.

Temperature °C	C g/dl	(Δh) mm water	$(\pi/C) \times 10^3$ atm dl/g	$A_2 \times 10^4$ ml mole/g ²
35.8	0.156	4.01	2.49	12.3
	0.312	9.53	2.96	
6.9	0.156	3.57	2.22	10.2
	0.312	8.40	2.61	

calculated from the slopes of the curves and have the same value within experimental error. This coefficient has frequently been reported to decrease with increasing molecular weight, but the picture is undoubtedly complicated by such factors as molecular heterogeneity etc.

The θ -point

For a polymer-solvent system, the θ -temperature is that at which the chemical potential due to segment-solvent interactions is zero and deviations from ideality vanish. The temperature $T = \theta$ for a polymer solution is thus analogous to the Boyle point of a real gas. The θ -point is characteristic of a given polymer-solvent pair and is independent of the molecular weight of the former. It may be determined from osmotic measurements as the temperature at which the second virial coefficient, A_2 , is zero. Additional osmotic measurements were thus made at the temperatures 35.8 and 6.9°C on fraction C10 and the data are given in Table 11. In Fig. 26 plots are

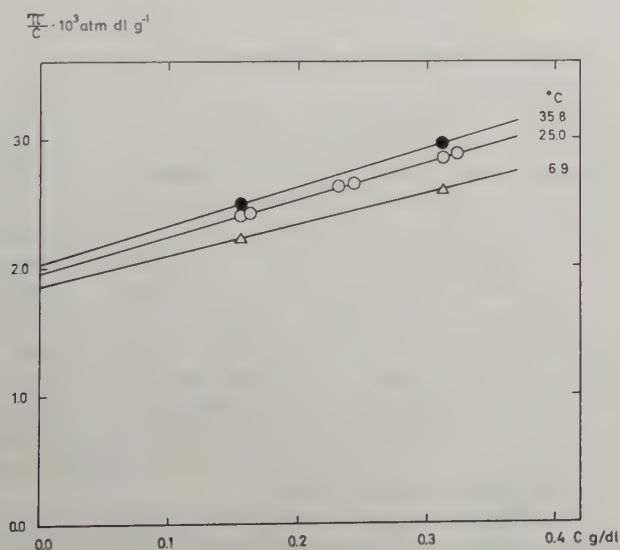


Fig. 26. Reduced osmotic pressure, (π/C) , as a function of concentration for hydroxyethyl cellulose fraction C10 at 35.8, 25.0, and 6.9°C.

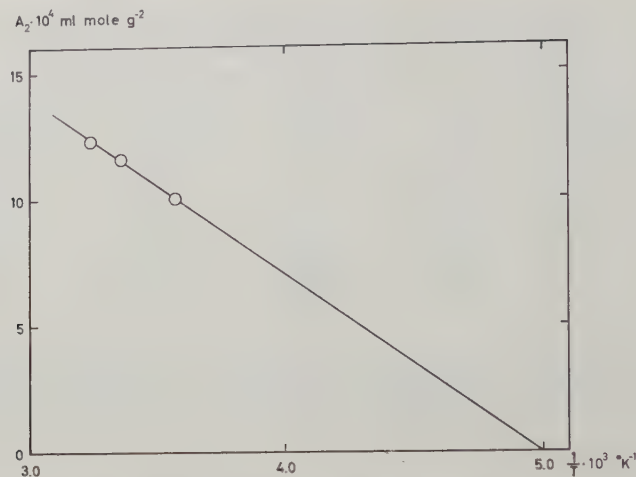


Fig. 27. Determination of the θ -point. The second virial coefficient, A_2 , plotted versus reciprocal absolute temperature for hydroxyethyl cellulose fraction C10.

shown of (π/C) vs C at these temperatures, and a small decrease in the slope with decreasing temperature is apparent. Values of A_2 were calculated from the slopes and are plotted against reciprocal absolute temperature in Fig. 27. The intersection of the curve with the temperature axis denotes the θ -point and corresponds to 200°K . Although there is a probability of a large error owing to the long extrapolation, the result is in keeping with that which one would expect for a water-soluble cellulose derivative.

10. Light scattering

Light scattering measurements on aqueous solutions of cellulose derivatives, as a means of determining molecular weights and dimensions, have often been of limited value as the solutions are particularly difficult to clean of suspended material. Dust particles are difficult to remove from water and it is also possible that they possess high refractive indices relative to this solvent. Fortunately, the scattering of the solvent is not a major source of error as its contribution to the intensity of the light scattered by the solution is deducted during treatment of the results. The most serious problem arises from the presence of insoluble, or partially soluble, solute material which is usually present with cellulose derivatives. Cellulose may be regarded as a partly crystalline and partly amorphous material, this structural inhomogeneity resulting in a derivative with a non-uniform distribution of substituent groups and a corresponding variation in the solubility. As the intensity of the light scattered by a particle increases with increasing volume, unremoved aggregated polymer can be a most disturbing factor in the measurements.

Manley [11] assumed such material to be present in aqueous solutions of ethyl hydroxyethyl cellulose and applied many techniques in an effort to remove it; for example, working in the presence of the hydrogen-bond breaking substance, urea, freezing the solution, ultrasonic vibrations, etc. Nevertheless, abnormally high

dissymmetries were still obtained. At that time, the most satisfactory method of removing the aggregated material was filtration under nitrogen pressure through as fine a membrane filter as possible without reducing the solution concentration. As the values of the molecular weight were still too high by a factor of two compared with the corresponding values from sedimentation equilibrium, the difference was attributed to a small amount of aggregated material able to pass through the filter.

In 1956, Dandliker and Kraut [36] described a method ("ultraclarification") by which solutions could be effectively cleaned of foreign material by centrifugation of the light scattering cell. Optical modifications to the apparatus have been subsequently suggested by Huque *et al.* [37]. This procedure eliminates the extreme precautions usually associated with light scattering measurements as the tedious transference of solutions is avoided. Depending on relative densities, the suspended matter sediments to the small tip of the cell under centrifugation or floats to the constricted neck; the cell assembly used in the present work is described in detail below.

Experimental procedures

Preparation of solutions

Stock solutions of hydroxyethyl cellulose fractions C2, C8, C9, and C10, in order of decreasing molecular weight, were prepared and cleaned as described in section 3. Solutions were prepared by dilution of the stock solutions and used for the refractive index increment and light scattering measurements.

Refractive index increment

As the intensity of scattering from a polymer solution is a function of the square of the rate of change of refractive index with concentration, $(dn/dC)^2$, this quantity must be accurately determined.

The apparatus used was a high-precision double-prism differential refractometer designed and constructed at this Institute. The instrument was calibrated for the wavelengths 546 $m\mu$ and 436 $m\mu$ with aqueous solutions of sodium sulphate; the reproducibility of these latter measurements was 1×10^{-6} . The relation between refractive index and concentration for aqueous sodium sulphate solutions was taken from the data of Kruis [49]. Values of dn/dC for the four hydroxyethyl cellulose fractions were measured at 25°C in the concentration range 0.1–0.4 g/dl at both wavelengths, and the results are presented in Table 12.

Table 12. Refractive index increment data.

Fraction	C g/dl	dn/dC ml/g	
		546 $m\mu$	436 $m\mu$
C 2	0.325	0.139	0.141
C 8	0.399	0.140	0.142
C 9	0.282	0.138	0.140
C 10	0.417	0.139	0.141
Mean		0.139	0.141

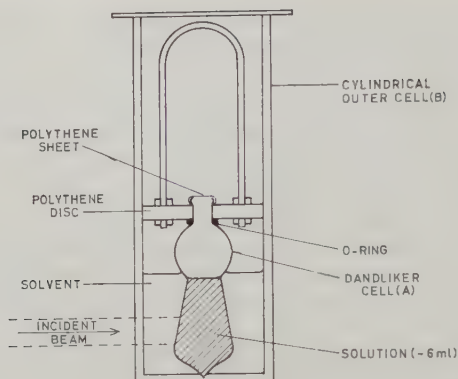


Fig. 28. Light scattering cell assembly.

Light scattering measurements

The light scattering measurements were made with a conventional type instrument similar to that described by Zimm [50] and constructed at this Institute by Dr. A. Bengtsson.¹ The absolute calibration of the apparatus was made by reflectivity measurements using a MgO surface having a reflectivity of 0.97 at 436 $m\mu$. As a secondary calibration standard, aqueous solutions of a dextran sample ($\bar{M}_w = 120,000$) were used. The light source was a medium pressure Luma 125 W mercury lamp and an unpolarized incident beam was used. A neutral filter was employed when measuring the intensity of the incident beam at 0°.

A sketch of the cell assembly is shown in Fig. 28. Cell *A* (Dandliker cell) was made from thin-walled Pyrex tubing and was free from imperfections on the conical surface; its pointed tip is centred in a hole in the base of the outer cell *B* and held in a vertical position by the holder. The holder consists of a thick polythene disc which is a "push fit" in the cell and has a hole in its centre fitting the top of the cell *A*; a convenient metal grip is attached to the disc. Cell *B* is cylindrical with plane parallel entrance and exit windows to facilitate measurements of the primary beam. Cell shields to prevent stray light entering the receiver were found to be of negligible advantage.

Each cell of type *A* was filled with solution (precleaned as in section 3) up to the neck as shown, and sealed with thin polythene sheet held in place by a hard rubber O-ring. Three such cells were then floated in glycerol in the buckets of the centrifuge rotor (Spinco ultracentrifuge, model L, rotor sw 25). Centrifugation was at 20,000 r.p.m. for 2 hours. On removal from the centrifuge each cell was carefully washed free of glycerol and mounted in cell *B* for measurement. The outer cell contained solvent in order to reduce reflection and refraction at the conical surface of cell *A*. Distilled water was doubly distilled in a quartz apparatus and used in the outer cell; its dissymmetry was approximately 1.1. The intensity of the scattered light was measured at 10° intervals over the range 45°–135° and the intensity of the incident beam at 0°, employing the blue mercury line 436 $m\mu$; the green mercury line (546 $m\mu$) gave the same values of KC/R_θ , indicating the absence of fluorescence. Measurements were

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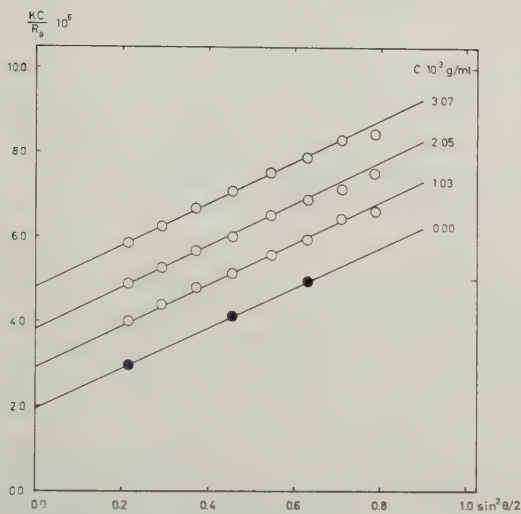


Fig. 29.

Fig. 29. (KC/R_θ) plotted versus $\sin^2 \theta/2$ for hydroxyethyl cellulose fraction C8 at several fixed concentrations in water.

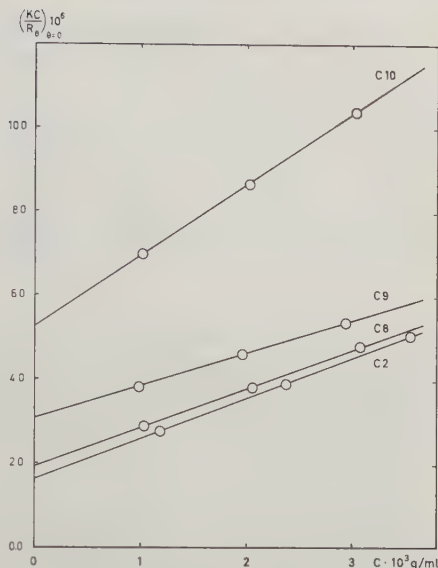


Fig. 30.

Fig. 30. Concentration dependence of $(KC/R_\theta)_{\theta=0}$ for fractions of hydroxyethyl cellulose in water.

made on solutions of fractions C2, C8, C9, and C10 at several concentrations in the range 0.1–0.4 g/dl at room temperature (approximately 22°C). The scattering of the solvent was also recorded over the same range of angles.

Dissymmetries, $[z_{45}]_{c=0} = (i_{45}/i_{135})_{c=0}$, are recorded in Table 13; the values at the different concentrations were too irregular to form the basis for molecular weight determination.

Each intensity ratio, (i_θ/i_0) , was corrected in the usual way, that is, multiplication by $(2 - \sin^2 \theta)^{-1}$ to take account of the horizontal component of the unpolarized incident light, multiplication by $\sin \theta$ to correct for the variation with angle of the scattering volume viewed by the receiver, and subtraction of the correspondingly corrected intensity of the solvent. Correction was made for refraction at the cell wall by multiplying by the square of the refractive index of the solution (assumed to equal the solvent refractive index) according to Hermans and Levinson [38]. This correction is valid irrespective of the shape of the scattering cell provided the receiver does not see past the edges of the beam; this was the case with the present apparatus. Values of KC/R_θ were calculated and plotted against $\sin^2 \theta/2$ where $K = 2\pi^2 n_s^2 (dn/dC)^2 / N_A \lambda^4$, n_s = solvent refractive index, dn/dC = refractive index increment, λ = wavelength in vacuum, θ = angle between scattered and transmitted beam, N_A = Avogadro's number, C = concentration in g/ml, and R_θ = Rayleigh ratio at angle θ (calculated from the corrected intensity ratio using the apparatus constants).

Such a plot is illustrated in Fig. 29 where the open circles represent data obtained at three concentrations of fraction C8 and the intercepts are the values at zero angle. Solutions centrifuged for an insufficient time showed pronounced curvature in these

Table 13. Summary of light scattering data.

Fraction	$[z_{45}]_{C=0}$	$\bar{M}_w \times 10^{-3}$	$\bar{Z}_w$	$(\bar{S}^2)_z^{\frac{1}{2}}$ Å	$A_2 \times 10^4$ ml mole/g ²
C 2	2.03	625	2650	750	4.7
C 8	1.84	515	2180	715	4.6
C 9	1.67	335	1420	565	3.9
C 10	1.20	190	805	450	8.5

plots; the satisfactory nature of the clarification is shown by the linearity of the curves. Fig. 30 shows the zero angle intercepts from Fig. 29 plotted versus concentration for the four fractions studied. According to the relationship

$$\frac{KC}{R_\theta} = \frac{1}{\bar{M}_w} + 2A_2C, \quad (30)$$

the reciprocals of the intercepts give the weight-average molecular weights and the slopes the second virial coefficient, A_2 . Molecular weights and second virial coefficients are given in Table 13, together with the weight average degree of polymerization, $\bar{Z}_w$ (the monomer molecular weight of the hydroxyethyl cellulose, $M.S. = 1.67$, is 236).

To determine the z -average root mean square radius of gyration, $(\bar{S}^2)_z^{1/2}$, data for constant angle were plotted versus concentration in the same way as the zero-angle data in Fig. 30 and the intercepts at zero concentration plotted against $\sin^2 \theta/2$ as

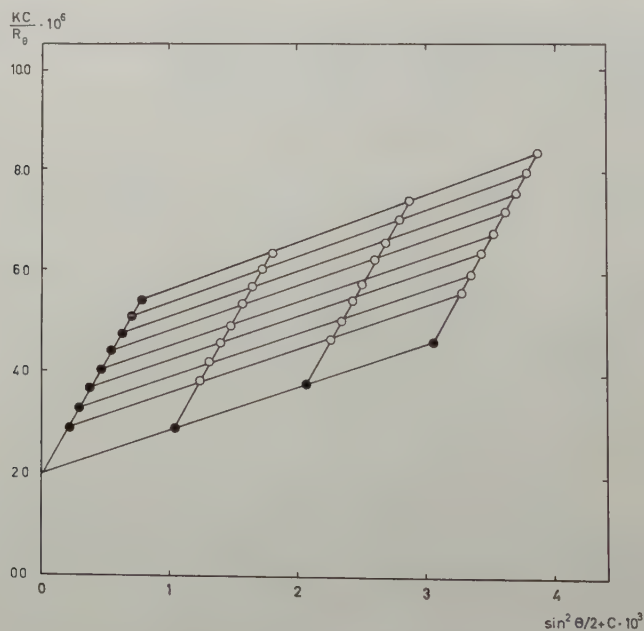


Fig. 31. Zimm plot for hydroxyethyl cellulose fraction C8.

shown by the filled circles in Fig. 29. $(\bar{S}^2)_z^{1/2}$ was determined for each fraction from the ratio of the slope to the intercept of this line, using the relation

$$\left(\frac{\text{Initial slope}}{\text{Intercept}} \right) = 16\pi^2/3 (\bar{S}^2)_z (n/\lambda)^2, \quad (31)$$

where λ/n is the wavelength of light in the solution of refractive index n ; $(\bar{S}^2)_z^{1/2}$ values are given in Table 13. Equivalent results may be obtained by plotting the data according to Zimm as shown in Fig. 31 for fraction C8.

Results and discussion

Using the Dandliker procedure of centrifuging the light scattering cell directly, it has been possible to obtain satisfactory data on the hydroxyethyl cellulose molecule in water solution, a summary of which is given in Table 13. However, as with light scattering the property being measured decreases with decreasing molecular weight, one may expect the figures obtained for fraction C10 to be the least reliable, as its molecular weight lies on the lower limit of usefulness of the method.

The weight average molecular weights, $\bar{M}_w$, are about 20 per cent higher than the corresponding figures of $\bar{M}_{wv}$ from sedimentation-diffusion given in Table 9. This difference is approximately that which might be expected between these two averages from the heterogeneities of the fractions, $\bar{M}_{wv}$ being an average dependent on the basis of selecting a mean position in the rather ill-defined sedimentation and diffusion boundaries which are broadened by molecular weight heterogeneity. For the purpose of introducing suitable corrections for this heterogeneity, it will be assumed that the fractions possess molecular weight distributions of the form [50]

$$f(N) = (y^{h+1}/h!) N^h e^{-yN}. \quad (32)$$

For this distribution

$$\bar{M}_z : \bar{M}_w : \bar{M}_n = (h+2) : (h+1) : h \quad (33)$$

Values of h and $\bar{M}_z$, calculated from $\bar{M}_w/\bar{M}_n$, are given in Table 14.

Determination of the angular variation of the scattered intensity affords a measure of the dimensions of the randomly coiled molecule in the form of the z -average radius of gyration according to equation (31). However, Zimm [50] and Benoit [44b] have shown that the mean square radius of gyration of the scattering centres is obtained

Table 14. Molecular parameters of hydroxyethyl cellulose in water solution.

Fraction	$\bar{M}_w/\bar{M}_n$	h	$\bar{M}_z \times 10^{-3}$ (calc)	$\bar{Z}_z$	$(\bar{R}_{\max}^2)_z \times 10^{-3}$ Å	$(\bar{R}^2)_{wv}^{\frac{1}{2}}$ Å	$(\bar{R}^2)_z^{\frac{1}{2}(a)}$ Å
C 2	1.64	1.56	870	3670	18.9	1450	1830
C 8	1.66	1.52	720	3040	15.7	1335	1750
C 9	1.78	1.28	480	2020	10.4	1150	1390
C 10	1.52	1.92	255	1075	5.55	775	1110

(^a) Calculated assuming Gaussian statistics.

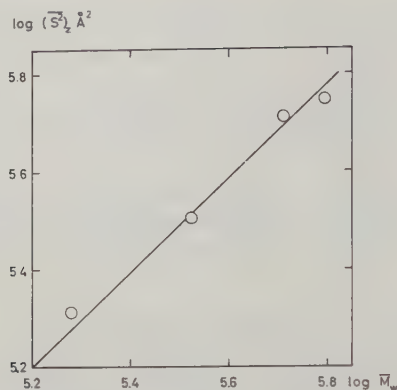


Fig. 32. Logarithm of the mean square z-average radius of gyration plotted against logarithm weight average molecular weight for hydroxyethyl cellulose fractions in water.

even if the molecule does not conform to one of the accepted simple models. In Fig. 32, $\log (\bar{S}^2)_z$ is plotted versus $\log M_w$, the equation of the line corresponding to

$$(\bar{S}^2)_z = 1.6 \bar{M}_w^{0.96}. \quad (34)$$

In good solvents, the square of the radius of gyration has been found to depend on molecular weight according to

$$(\bar{S}^2) = K M^{1+\varepsilon}. \quad (35)$$

From equation (34), $\varepsilon = -0.04$ for hydroxyethyl cellulose compared to $\varepsilon = 0.33$ for cellulose trinitrate [75]. The difference in ε for these two compounds indicates the comparatively low extension of hydroxyethyl cellulose compared to cellulose trinitrate.

Assuming the Gaussian coil model, $(\bar{S}^2)_z^{1/2}$ is related to the z-average root mean square end-to-end distance of the molecule by the relation

$$(\bar{R}^2)_z^{1/2} = 6^{1/2} (\bar{S}^2)_z^{1/2}. \quad (36)$$

Values of $(\bar{R}^2)_z^{1/2}$ calculated in this way are given in Table 14. The weight-weight average end-to-end distances $(\bar{R}^2)_{ww}^{1/2}$ were calculated from $(\bar{R}^2)_z^{1/2}$ employing the ratio $\bar{M}_{ww}/M_z$ and are also listed in Table 14. $(\bar{R}^2)_{ww}^{1/2}$ may be compared directly with $(\bar{R}^2)_{ww}^{1/2}$ calculated from various hydrodynamic theories presented in the following section (Table 21).

The second virial coefficient, A_2 , is usually found to decrease slowly with increasing molecular weight. As seen in Table 13, the values for fractions C2, C8 and C9 are the same within experimental error, while fraction C10 has a significantly higher value of A_2 in agreement with the expected trend. It may be noted that irregularities in A_2 have often been reported; for example, Huque *et al.* [45] for cellulose trinitrate. The values of A_2 obtained from osmotic pressure measurements and given in Table 10 are significantly higher than the values from light scattering. This has been frequently found to be the case [11, 72] and is probably due to the sensitivity of A_2 to the molecular weight heterogeneity of the sample [71].

Table 15. Configurational parameters for hydroxyethyl cellulose in water solution.

Fraction	$q_{\Phi'}$	$\Phi' \times 10^{-22}$	α	$(\overline{S_0^2})_z^{\frac{1}{2}}$ Å	$(\overline{S_0^2}/\overline{M})_z$ Å ² /g mole	$\frac{(\overline{R_{\max}})_z}{(\overline{R^2})_z^{\frac{1}{2}}}$	b Å
C 2	1.725	2.72	1.04	720	0.59	10.3	29.1
C 8	1.735	2.18	1.03	695	0.66	9.0	30.7
C 9	1.820	2.55	1.02	555	0.64	7.5	30.0
C 10	1.620	1.29	1.03	435	0.73	5.0	32.5

The relation of Flory [40] describes the dependence of the intrinsic viscosities of most flexible chain polymers on their molecular dimensions, according to

$$[\eta] = \Phi' (\overline{S_0^2})^{3/2} \overline{M}^{1/2} \alpha^3, \quad (37)$$

where $(\overline{S_0^2})^{1,2}$ is the radius of gyration in the absence of long-range interactions and α is the molecular expansion factor. For randomly coiling polymers Φ' has a constant value independent of solvent and polymer, but for many cellulose derivatives Φ' depends on molecular weight and only at high molecular weight does it approach the asymptotic constant value ordinarily found. This is explained by the high extension and stiffness of cellulose derivatives in the low molecular weight region, Gaussian statistics only being approached at high degrees of polymerization if the long-range interactions are small. Alternatively, one may suggest that the variation in Φ' arises from a change in the permeability of the molecule with change in molecular weight. Such behaviour is shown by cellulose trinitrate [41], cellulose tricaproate [42] and ethyl hydroxyethyl cellulose [11] amongst others. To evaluate Φ' from experimental data, we may rewrite equation (37)

$$\Phi' = \frac{q_{\Phi'} [\eta] \overline{M}_w}{(\overline{S^2})_z^{3/2}}, \quad (38)$$

where $(\overline{S^2})_z$ has replaced $\alpha^2 (\overline{S_0^2})$ and a factor $q_{\Phi'}$ introduced to take account of polydispersity [43], where

$$q_{\Phi'} = \frac{(\langle \overline{S^2} \rangle_z)^{3/2} \langle M \rangle_n}{\langle (\overline{S^2})^{3/2} \rangle_n \langle M \rangle_w}. \quad (39)$$

The correction factor $q_{\Phi'}$ has been discussed in detail by Krigbaum and Carpenter [39]. Assuming the above distribution given in equation (32), $q_{\Phi'}$ is expressed by

$$q_{\Phi'} = \frac{(h+2)^{3/2} \Gamma(h+2)}{(h+1)^2 \Gamma(h+1.5)}, \quad (40)$$

where $\Gamma(h)$ is the gamma function of (h) . Using equation (40), $q_{\Phi'}$ has been calculated from the h -values in Table 14 and the values are listed in Table 15. Values of Φ' were then calculated from equation (38) and are given in Table 15; Φ' increases with increasing molecular weight and for the highest fraction has nearly reached the asymptotic value of 3.2×10^{22} ($[\eta]$ expressed in dl/g) found for most non-cellulosic polymers.

Flory [40] assumed a uniform coil expansion measured by the factor

$$\alpha = (\overline{S^2})^{1/2} / (\overline{S_0^2})^{1/2}. \quad (41)$$

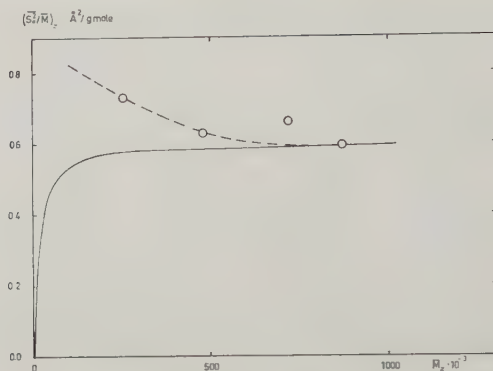


Fig. 33. Dependence of $(\overline{S}_0^2/\overline{M})_z$ on $\overline{M}_z$ for hydroxyethyl cellulose fractions in water; (O) experimental results; (---) experimental curve; (—) theoretical curve calculated from equation (44 a) for $q = 83 \text{ \AA}$.

Values of the molecular expansion factor α , given in Table 15, were calculated from the observed quantities A_2 , $\overline{M}_w$ and $(\overline{S}_z^2)^{1/2}$ using the approximate relation due to Orofino and Flory [47]

$$A_2 = \left(\frac{16\pi}{3^{3/2}} \right) (N_A (\overline{S}_z^2)^{3/2} / \overline{M}_w^2) \ln [1 + (\pi^{1/2}/2)(\alpha^2 - 1)]. \quad (42)$$

The values of α are effectively constant and are close to unity indicating that long-range interactions have a negligible effect on the configuration in the molecular weight interval considered here. Similar results have been reported for cellulose trinitrate [41]. It should be noted, however, that equation (42) is dependent on assumptions on the coil expansion (which may be uniform or non-uniform) and so the absolute magnitude of α should be treated with reserve.

The effective bond length, b , is given by the expression

$$b = (6\overline{S}_0^2/\overline{Z})^{1/2}, \quad (43)$$

where $\overline{Z}$ is the degree of polymerization, and the values are given in Table 15. b decreases with increasing molecular weight to about $29 \text{ \AA}$ in the asymptotic region; the corresponding value for cellulose trinitrate has been reported to be $35 \text{ \AA}$ [41]. The effective bond length is considered in more detail in the following section.

Another useful quantity for characterizing the stiffness of the chain is the persistence length, q , introduced by Kratky and Porod [68, 69]. It equals the mean length of projection of an infinitely long chain along the direction of the first link. Benoit and Doty [74] derived the following equation between $\overline{S}_0^2$ and q

$$\overline{S}_0^2 = q^2 [(x/3) - 1 + (2/x) - 2(1 - e^{-x})/x^2], \quad (44 a)$$

where x is the number of Kratky-Porod lengths defined as

$$x = \overline{R}_{\max}/q, \quad (44 b)$$

and $\overline{R}_{\max}$ is the contour length of the molecule obtained as the product of the degree

of polymerization and the length of the anhydroglucose unit (5.15 Å). When x is large, equation (44a) may be written

$$S_0^2 = \bar{R}_{\max}^2(q/3), \quad (44\ c)$$

and one obtains for the highest molecular weight fraction $q = 83$ Å. The corresponding value for cellulose trinitrate is 117 Å [41]. It can be concluded that the hydroxyethyl cellulose molecule is appreciably less stiff in the high molecular weight region than the cellulose trinitrate molecule, but significantly stiffer than most non-cellulosic polymer molecules which have q -values in the region 8–10 Å. The segment length of the Kuhn equivalent chain $A_m = 2 q$, (discussed in the next section) is 166 Å. The segment length of the Kuhn chain thus consists of rod-like sequences of 32 anhydroglucose units joined to each other without valence angle restrictions.

The ratio $(\bar{S}_0^2/\bar{M})_z$ is given for the four fractions in Table 15 and plotted versus $\bar{M}_z$ in Fig. 33. It shows a tendency to increase with decreasing molecular weight as shown by the dotted line and indicates the high extension of the molecule in the low molecular weight region and departure from random statistics. Similar behaviour has been reported by Krigbaum and Sperling for cellulose tricaproate [42] and Cowie for amylose [46]. The theoretical curve for the dependence of $(\bar{S}_0^2/\bar{M})$ on $\bar{M}$ was calculated using the value of $q = 83$ Å in equation (44a) and is represented by the continuous curve in Fig. 33. This predicts non-Gaussian behaviour below $\bar{M}_z \approx 300,000$ but with opposite curvature to that indicated experimentally. Adherence to the theoretical curve has, however, been reported for cellulose trinitrate [41].

The extended nature of the molecule, which is particularly evident at low molecular weight, is thus caused chiefly by short-range interaction forces in addition to swelling of the molecule by polymer-solvent interaction, etc. For linear polymers we can obtain a measure of the steric hindrance to rotation in the chain using the expression

$$\bar{R}_0^2 = Zb_0^2 \left(\frac{1 - \cos \theta}{1 + \cos \theta} \right) \left(\frac{1 + \overline{\cos \phi}}{1 - \overline{\cos \phi}} \right) \quad (45)$$

where b_0 is the length of the anhydroglucose unit, 5.15 Å, and θ is the oxygen valence angle ($\approx 110^\circ$) for cellulose and its derivatives [48]. The mean value of $\cos \phi$ for the fractions is 0.89; $\cos \phi = 0$ corresponds to free rotation about the C–O bonds linking the pyranose rings in the chain, and $\cos \phi = 1$ to the absence of rotational freedom and maximum extension of the molecule. The hydroxyethyl cellulose molecule is thus obviously sterically hindered to a great degree as regards rotation, giving rise to the pronounced molecular extension at low molecular weights. This hindrance may be caused partly by interaction of the hydroxyethyl side groups, each of which is approximately the same length as an anhydroglucose unit, in addition to the basic stiffness of the cellulose chain. Our knowledge of the configurational properties of the cellulose molecule is at present rather limited. However, Henley [67] has studied cellulose in the complex solvent *cadoxen* and found that, although substantially stiffer than most polymers, the cellulose molecule is significantly more flexible in this solvent than the hydroxyethyl cellulose molecule in water. For example, the effective bond length $b = 19$ Å and the Kuhn equivalent chain segment length $A_m = 70$ Å.

The reciprocal extension ratio

$$(\bar{R}_{\max})_z/(\bar{R}^2)_z^{1/2}, \quad (46)$$

is a direct measure of the degree of coiling of the molecule. For values exceeding 10, the spatial arrangement of the molecule should be approximately spherically symmetric [43] and the molecule exhibit the behaviour of a randomly coiled chain polymer. Values of this ratio are given in Table 15 and it increases with increasing molecular weight to a value of about 10 for the highest fraction. This indicates that for $\bar{M}_w > 600,000$ the behaviour of the hydroxyethyl cellulose molecule should approach that of a flexible chain polymer, which is in good agreement with the increasing value of Φ' , discussed above, towards an asymptotic limit at this molecular weight.

It may be noted here that the ratios of molecular length to diameter obtained from the frictional ratio and given in Table 9, are of the correct order of magnitude. The ratio l/d is derived from the frictional ratio assuming the molecule to be unhydrated and have an ellipsoidal form. Using the values of $(R^2)_{ww}^{1/2}$ calculated from $(\bar{R}^2)_z^{1/2}$, the hydrodynamic thickness of the molecule is found to be $\approx 8 \text{ \AA}$, in reasonable agreement with the expected value.

11. The molecular configuration from hydrodynamic behaviour

A linear polymer molecule in dilute solution may be considered to have the form of a loosely coiled chain, through which the flow of solvent is retarded to an extent dependent on its structure and size. For a free draining coil, $[\eta]$ is proportional to the degree of polymerization Z , s is independent of Z , and D is proportional to Z^{-1} , whereas if the flow of solvent is totally hampered, $[\eta]$ is proportional to $Z^{1/2}$, and s and D are proportional to $Z^{1/2}$ and $Z^{-1/2}$ respectively. Most linear polymers in dilute solution exhibit a behaviour between these extremes and existing theories consider the hydrodynamic behaviour of the molecule for the intermediate case of partial solvent immobilization and derive relationships for the intrinsic viscosity and the sedimentation and diffusion coefficients. The theories of Kirkwood and Riseman, Kuhn and Kuhn, and Peterlin, basically assume the linear relation

$$(\bar{R}^2)^{1/2} = b Z^{1/2} \quad (47)$$

between the R.M.S. end-to-end distance and $Z^{1/2}$, and make use of linear plots of $Z/[\eta]$ vs $Z^{1/2}$, s vs $Z^{1/2}$, and DZ vs $Z^{1/2}$; from the slopes and intercepts the linear dimensions of the coil can be determined. Such plots for the viscosity, sedimentation and diffusion data, collected in Table 16, are shown in Figs 34, 35, and 36; the equations of the lines are given by

$$[\eta] = \left(\frac{\bar{Z}_{ww}}{0.66 + 0.032 Z_{ww}^{1/2}} \right) \times 10^{-2}, \quad (48)$$

$$s_0 = (0.20 + 0.11 \bar{Z}_{ww}^{1/2}) \times 10^{-13}, \quad (49)$$

$$D_0 \bar{Z}_{ww} = (0.80 + 0.39 \bar{Z}_{ww}^{1/2}) \times 10^{-5}. \quad (50)$$

For the viscosity data, the Kirkwood-Riseman theory uses the closely related $[\eta]/Z^{1/2}$ vs $1/Z^{1/2}$ plot, shown in Fig. 37. The Debye-Bueche and Brinkman theory employs

Table 16. Molecular weights and degrees of polymerization, sedimentation and diffusion coefficients, and intrinsic viscosities at 25°C.

Fraction	$\bar{M}_{ww} \times 10^{-3}$	$\bar{Z}_{ww}$	$s_0 \times 10^{13}$	$D_0 \times 10^7$	$[\eta]_{25}$ dl/g
C 2	545	2310	5.65	0.85	10.62
C 8	420	1780	4.90	0.96	8.95
C 9	330	1400	4.45	1.11	7.54
C 10	125	530	2.82	1.86	3.81

the exponents of equations of the form $[\eta] = AZ^{\epsilon_1}$ and $f = BZ^{\epsilon_2}$, where f is the frictional coefficient.

It is interesting to see to what extent the hydroxyethyl cellulose molecule fits into the framework of these theories and in what respects it deviates from the expected behaviour of an ideal flexible chain molecule. The hydrodynamic theories are briefly outlined below and used to calculate the R.M.S. end-to-end distances from $[\eta]$, s_0 and D_0 measured on the hydroxyethyl cellulose fractions; the values are compared with values of $(R^2)_{ww}^{1/2}$ calculated from $(R^2)_z^{1/2}$ from the light scattering measurements (Table 14).

Kirkwood and Riseman [51] treated the molecule on the basis of the ideal random coil, taking account of the hydrodynamic interaction of the monomer units and of the inhibited flow of solvent through the coiled chain; this is the well known "pearl

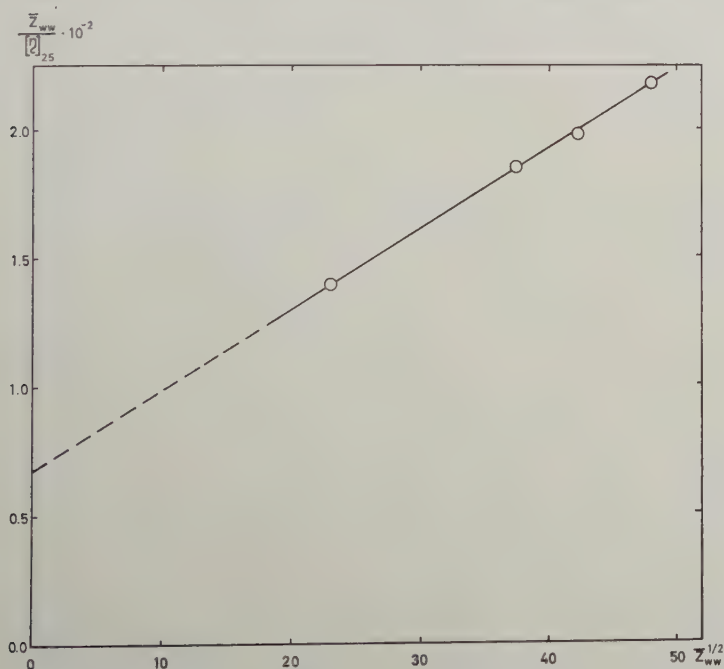


Fig. 34. $\bar{Z}_{ww}/[\eta]$ vs $Z_{ww}^{1/2}$ for hydroxyethyl cellulose fractions in water, where $\bar{Z}_{ww}$ is the weight-weight average degree of polymerization and $[\eta]$ the intrinsic viscosity.

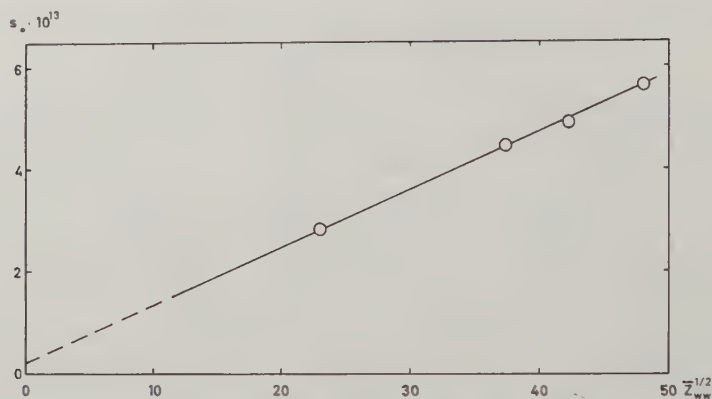


Fig. 35. Sedimentation constants at infinite dilution versus the square root of the degree of polymerization for hydroxyethyl cellulose fractions in water.

necklace" model. They introduced two parameters, b the effective bond length, and ξ the frictional coefficient per monomer unit. The former quantity is the bond length of a hypothetical, freely jointed chain having the same number of bonds and hence a much greater contour length than the actual chain; b thus provides a convenient measure of the stiffness of the molecule. Using the following equations for the experimental data, b and ξ may be calculated from viscosity and sedimentation-diffusion data using graphical procedures;

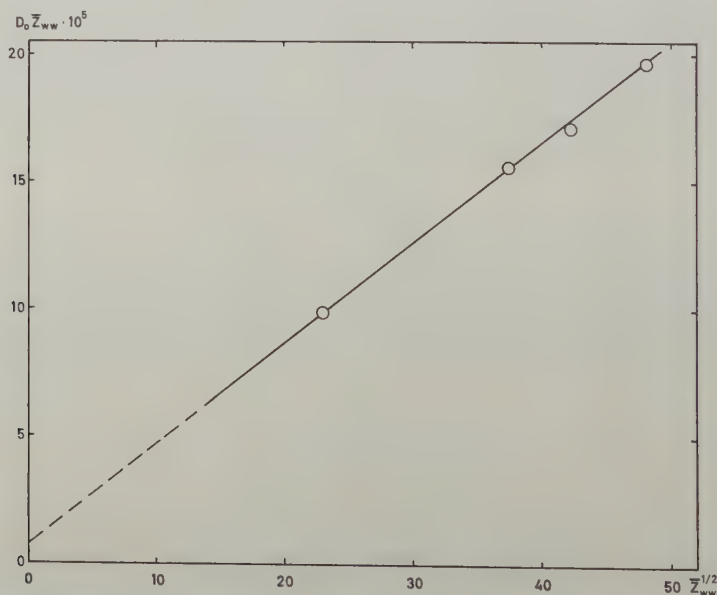


Fig. 36. $D_0 \bar{Z}_{ww}$ vs $\bar{Z}_{ww}^{1/2}$ for hydroxyethyl cellulose fractions in water, where $\bar{Z}_{ww}$ is the weight-average degree of polymerization and D_0 the diffusion coefficient at infinite dilution.

Table 17 a. Molecular parameters from the Kirkwood-Riseman theory.

	Sedimentation- diffusion	Viscosity
Effective bond length b , Å	23.3	27.2
Frictional constant per monomer unit $\xi \times 10^{10}$ g/sec	55	—
$\lambda_0 \times 10^2$	19.5	—

$$b^3 = \frac{2435 M_0}{(6\pi^3)^{1/2} N_A} \lim_{z \rightarrow \infty} \left(\frac{[\eta]}{\bar{Z}_{ww}^{1/2}} \right), \quad (51)$$

$$s_0 = \frac{M_0(1 - V\rho)}{N_A \xi} \left[1 + \frac{8}{3} \lambda_0 \bar{Z}_{ww}^{1/2} \right], \quad (52)$$

$$D_0 \bar{Z}_{ww} = \frac{kT}{\xi} \left[1 + \frac{8}{3} \lambda_0 \bar{Z}_{ww}^{1/2} \right], \quad (53)$$

where M_0 = molecular weight of the monomer unit, N_A = Avogadro's number, $[\eta]$ = intrinsic viscosity in units of dl/g throughout this section, V = partial specific volume of solute, ρ = solution density, λ_0 = constant for the polymer-solvent system, k = Boltzmann's constant, and T = temperature in °K.

The frictional coefficient per monomer unit, ξ , is related to the effective bond length, b , by

$$\xi = \lambda_0 [(6\pi^3)^{1/2} \eta_0 b], \quad (54)$$

where η_0 is the solvent viscosity.

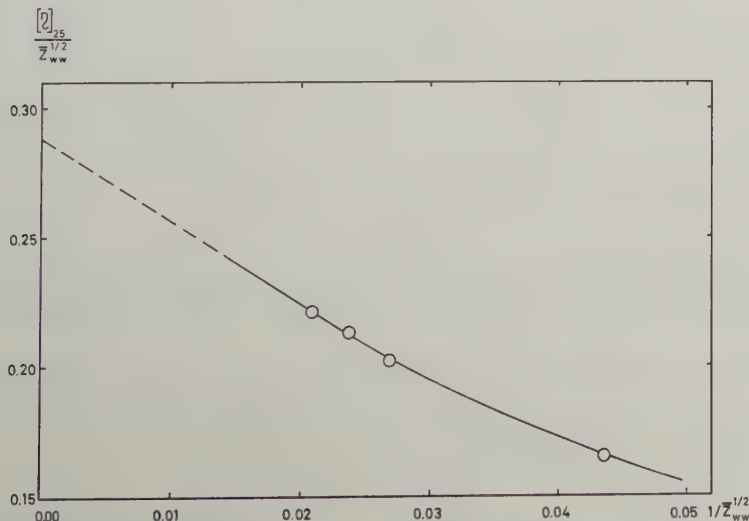


Fig. 37. Plot of $[\eta]/\bar{Z}_{ww}^{1/2}$ vs $1/\bar{Z}_{ww}^{1/2}$, according to Kirkwood-Riseman, for hydroxyethyl cellulose fractions in water, where $\bar{Z}_{ww}$ is the weight-weight average degree of polymerization and $[\eta]$ the intrinsic viscosity.

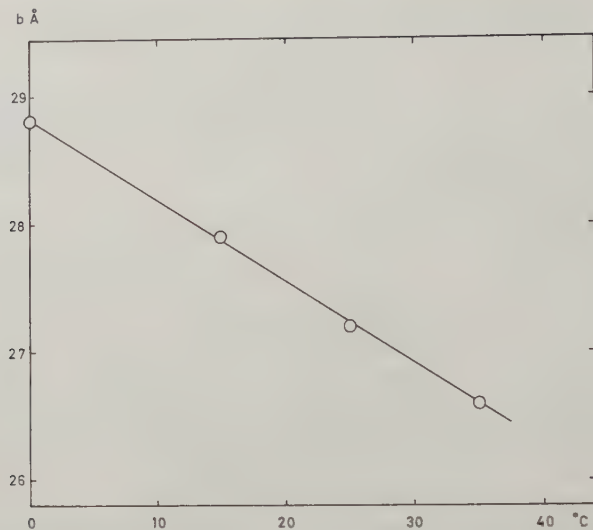


Fig. 38. The variation of the effective bond length, b , with temperature for hydroxyethyl cellulose fractions in water; b has been calculated from the viscosity data according to the Kirkwood-Riseman theory.

The parameters given in Table 17*a* have been obtained from equations (51), (52), and (53), using the intercept from Fig. 37, and the slopes and intercepts from Figs 35 and 36 given in equations (49) and (50). The parameter b was also estimated at different temperatures from the intrinsic viscosities by similar plots to Fig. 37; the values are given in Table 17*b*.

In Fig. 38, b is plotted versus temperature and is seen to decrease linearly with increasing temperature. A significantly higher value of b is obtained from viscosity compared to that from sedimentation-diffusion data. Values in the literature include $b = 35$ Å for the stiff cellulose trinitrate molecule [41], and $b = 7.7$ Å for the relatively flexible polystyrene molecule [53]; it can thus be concluded that the hydroxyethyl cellulose molecule is highly extended. The R.M.S. end-to-end distance may be calculated from the b -values using the relation

$$(\bar{R}^2)^{1/2} = bZ^{1/2} \quad (55)$$

The values obtained from the viscosity and sedimentation-diffusion data are presented in Table 21. The viscosity data give values of $(\bar{R}^2)_{ww}^{1/2}$ in good agreement with

Table 17 *b*. The variation of the effective bond length with temperature.

Temperature, °C	b , Å
0	28.8
15	27.9
25	27.2
35	26.6

those calculated from light scattering, while $(\bar{R}^2)_{w,w}^{1,2}$ from sedimentation-diffusion are appreciably lower.

The Kirkwood-Riseman theory gives $\xi = 55 \times 10^{-10}$ gsec⁻¹ for the frictional coefficient per monomer unit. Using Stokes' law, this value corresponds to a monomer unit hydrodynamic radius of 2.9 Å, which is of the correct order of magnitude.

Kuhn and Kuhn [54] considered the polymer molecule as a random coil formed of straight-line segments of length A_m and hydrodynamic thickness d_h , linked statistically to each other in a chain so that each segment is independent of the orientations of its neighbours. This chain has the same contour length as the actual molecule and thus a smaller number of bonds. Using large-scale wire models these authors carried out experiments to investigate the hydrodynamic behaviour of such molecules and deduced the following relations; the modified constants are those given by Kuhn, Kuhn, and Silberberg [55].

$$[\eta] = \frac{N_A b_0^2 A_m}{100 M_0} \cdot \left[\frac{0.43 Z}{-1.6 + 2.3 \log_{10} (A_m/d_h) + (Zb_0/A_m)^{1/2}} \right], \quad (56)$$

$$s_0 = \frac{M_0(1 - V\rho)}{N_A b_0 \gamma_0} \cdot [-0.03 + 0.16 \log_{10} (A_m/d_h) + 0.136(Zb_0/A_m)^{1/2}], \quad (57)$$

$$D_0 Z = \frac{kT}{\eta_0 b_0} [-0.03 + 0.16 \log_{10} (A_m/d_h) + 0.136(Zb_0/A_m)^{1/2}], \quad (58)$$

where b_0 ($= 5.15$ Å) is the length of the glucose residue and the other quantities are as previously defined. On substituting the slopes and intercepts from equations (48) to (50) into the respective equations (56) to (58), values of A_m and d_h are obtained and are given in Table 18. The segment length of the Kuhn equivalent chain, A_m , is related to the R.M.S. end-to-end distance by

$$(\bar{R}^2) = A_m R_{\max}, \quad (59)$$

where $\bar{R}_{\max}$ is the average contour length of the molecule equal to $b_0 \bar{Z}$; values of $(\bar{R}^2)_{w,w}^{1,2}$ are given in Table 21.

The parameter A_m provides a useful measure of the flexibility of the chain and particularly the short-range interactions influencing the segment length. Values are given in Table 19 for hydroxyethyl cellulose, together with those of some other polymer-solvent systems for comparison, and range from 33 Å for the flexible polystyrene molecule to 234 Å for the stiff cellulose trinitrate molecule. The value of $A_m = 182$ Å from viscosity data corresponds to about 35 glucose residues per equi-

Table 18. Molecular parameters from the Kuhn-Kuhn theory.

	Sedimentation- diffusion	Viscosity
Equivalent chain segment length A_m , Å	51	182
Hydrodynamic thickness, d_h , Å	9.9	1.1

Table 19. Values of the Kuhn equivalent chain segment length, A_m , for hydroxyethyl cellulose in water and some other systems.

Polymer	Solvent	A_m Å
Hydroxyethyl cellulose	Water	182 (viscosity)
		51 (sedimentation-diffusion)
		176 (light scattering) ^a
Ethyl hydroxyethyl cellulose (ref. [11])	Water	216 (viscosity)
		120 (sedimentation-diffusion)
Cellulose trinitrate (ref. [55]) (ref. [41])	Acetone	193 (viscosity)
	Ethyl acetate	234 (light scattering)
Cellulose acetate (ref. [55])	Acetone	114 (viscosity)
Cellulose (ref. [67])	Cadoxen	147 (viscosity)
		107 (light scattering) ^a
Polystyrene (ref. [55])	Benzene	33 (viscosity)

^a Taking account of Flory's expansion factor, α .

valent chain segment length, while $A_m = 51$ Å from the sedimentation-diffusion data corresponds to about 10 glucose residues per chain segment. Manley [11] reported the value $A_m = 120$ Å for ethyl hydroxyethyl cellulose from sedimentation-diffusion data; however, if A_m is calculated from his viscosity results a value of 216 Å is obtained. Similar differences between A_m from viscosity and sedimentation-diffusion have been described [55, 60]. From the excellent agreement between A_m (viscosity) and A_m (light scattering) it is seen that A_m (sedimentation-diffusion) is substantially too low. The difference between A_m (viscosity) for hydroxyethyl cellulose and A_m (viscosity) for ethyl hydroxyethyl cellulose may be correlated with the difference in the exponents α of the modified Staudinger equation which are 0.7 and 0.8 respectively. From A_m (viscosity) for hydroxyethyl cellulose it is concluded that this molecule is less extended than ethyl hydroxyethyl cellulose and cellulose trinitrate but considerably more extended than cellulose acetate and polystyrene.

The value of the hydrodynamic thickness of the molecule, $d_h = 1.1$ Å, from viscosity data is unrealistic, as it is known to be about 10 Å for cellulose and its derivatives. However, the value of $d_h = 9.9$ Å from sedimentation-diffusion data is of the expected order of magnitude.

The Kuhn-Kuhn theory thus does not give a wholly satisfactory description of the hydroxyethyl cellulose molecule, the viscosity data providing a segment length and hence an end-to-end distance in substantial agreement with that from light scattering measurements, while the sedimentation-diffusion data give a realistic value of the hydrodynamic thickness of the molecule.

Debye, Bueche and Brinkman [58, 59] replaced the randomly coiled macromolecule by a hydrodynamically equivalent sphere containing a uniform segment density. The radius of this equivalent sphere is related to the R.M.S. end-to-end distance by the relation

$$2R_s = 1.054(\bar{R}^2)^{1/2}. \quad (60)$$

In terms of the experimentally obtained intrinsic viscosity and sedimentation and diffusion data, $(\bar{R}^2)_{ww}^{1/2}$ is given by the relationships

$$(\bar{R}^2)_{ww}^{1/2} = \frac{1}{1.054} \left(\frac{6}{N_A \Phi_\sigma \pi} \right)^{1/3} (100[\eta] \bar{M}_{ww})^{1/3}, \quad (61)$$

$$(\bar{R}^2)_{ww}^{1/2} = \frac{(1 - V_Q) \bar{M}_{ww}}{6\pi N_A 0.527 \eta_0 \Psi_\sigma \sigma^2 s_0}, \quad (62)$$

and

$$(\bar{R}^2)_{ww}^{1/2} = \frac{kT}{6\pi 0.527 \eta_0 \Psi_\sigma} \cdot \frac{1}{D_0}, \quad (63)$$

where Φ_σ and Ψ_σ are volume factors dependent on the shielding ratio σ , and the other symbols are as previously defined. These authors have tabled values of the shielding ratio and volume factors in terms of the exponents ε_1 and ε_2 of the equations

$$(a) [\eta] = K \bar{M}^{\varepsilon_1}, \quad (b) D_0 = K_D \bar{M}^{-\varepsilon_2}, \quad \text{and} \quad (c) s_0 = K_s \bar{M}^{(1-\varepsilon_2)}. \quad (64)$$

From equations (23), (24), and (25), $\varepsilon_1 = 0.70$ and $\varepsilon_2 = 0.54$; the corresponding values of Φ_σ and Ψ_σ are 1.22 and 0.87 respectively. Values of $(\bar{R}^2)_{ww}^{1/2}$ were calculated by substituting these factors in equations (61) to (63) and are given in Table 21. There is reasonable agreement between $(\bar{R}^2)_{ww}^{1/2}$ values from the viscosity data and those calculated from light scattering data but a large difference between these values and those from the sedimentation-diffusion data. This discrepancy may be partly due to the deviation of the hydroxyethyl cellulose molecule from the ideal Gaussian coil and partly due to the unrealistic model.

Peterlin [60] considered the hydrodynamic problems associated with the configuration of coiled macromolecules in dilute solution and suggested that from the slope of an $\bar{M}/[\eta]$ vs $\bar{M}^{1/2}$ plot, the R.M.S. end-to-end distance could be obtained from a relation of the form

$$(\bar{R}^2)^{1/2} = 1.52 \times 10^{-8} (100 \cot \alpha)^{1/3} \bar{M}^{1/2}, \quad (65)$$

where $\tan \alpha$ is the slope of the $\bar{M}/[\eta]$ against $\bar{M}^{1/2}$ plot; the value of $\tan \alpha$ may also be easily obtained from Fig. 34 ($\bar{M}_{ww} = M_0 \bar{Z}_{ww}$). The relationship between $(\bar{R}^2)_{ww}^{1/2}$ and $\bar{M}_{ww}^{1/2}$ is

$$(\bar{R}^2)_{ww}^{1/2} = 1.96 \times 10^{-8} \bar{M}_{ww}^{1/2}. \quad (66)$$

The R.M.S. end-to-end distances obtained from equation (66) are given in Table 21 *a*, where it is seen that they are in excellent agreement with the values of $(\bar{R}^2)_{ww}^{1/2}$ from light scattering.

Flory and Fox [56] related the intrinsic viscosity of a linear polymer molecule in solution to the ratio of the volume occupied by the molecule divided by the molecular weight, taking the volume of the molecule as the cube of the R.M.S. end-to-end distance

$$[\eta] = \Phi (\bar{R}_0^2 / M)^{3/2} M^{1/2} \alpha^3, \quad (67)$$

where $\alpha = (\bar{R}^2 / \bar{R}_0^2)^{1/2}$ is the expansion factor arising from long-range interactions

Table 20. End-to-end distances $(\bar{R}^2)_{ww}^{1/2}$, and the parameter $(\Phi^{1/2}P^{-1})$, from the Flory-Fox-Mandelkern theory.

Fraction	$\bar{M}_{ww} \times 10^{-3}$	$(\bar{R}^2)_{ww}^{1/2}$ Å ^(a) Light scattering	$(\bar{R}^2)_{ww}^{1/2}$ Å		$(\Phi^{1/2}P^{-1}) \times 10^{-6}$ ^(b)
			Viscosity	Sedimentation- diffusion	
C 2	545	1450	1405	1060	3.31
C 8	420	1335	1210	940	3.28
C 9	330	1150	1060	810	3.35
C 10	125	775	610	490	3.14

^(a) Calculated from $(\bar{R}^2)_z^{1/2}$.^(b) Uncorrected for molecular heterogeneity.

between molecular segments, $(R_0^2)^{1/2}$ is the "unperturbed" end-to-end distance, and Φ is a "constant" for all polymers irrespective of solvent ($\Phi \approx 2.1 \times 10^{21}$). In terms of the experimental data and assuming monodispersity,

$$(\bar{R}^2)_{ww}^{1/2} = \left(\frac{1}{\Phi}\right)^{1/3} ([\eta]\bar{M}_{ww})^{1/3} = 7.8 \times 10^{-8} ([\eta]\bar{M}_{ww})^{1/3} \quad (68)$$

Mandelkern and Flory [57] have related the frictional coefficient of the molecule to its R.M.S. end-to-end distance by the equation

$$f_0/\eta_0 = P(R_0^2/M)^{1/2} M^{1/2} \alpha, \quad (69)$$

where P is analogous to Φ in equation (67), and has the value 5.1. Expressing $(\bar{R}^2)^{1/2}$ in terms of the sedimentation and diffusion coefficients, we have for the experimental data

$$(\bar{R}^2)_{ww}^{1/2} = \frac{M_{ww}(1 - V\rho)}{P\eta_0 N_A s_0} \quad (70)$$

and

$$(\bar{R}^2)_{ww}^{1/2} = \frac{kT}{P\eta_0 D_0}, \quad (71)$$

Table 21 a. End-to-end distances from viscosity data compared with the corresponding values from light scattering measurements.

Fraction	$(\bar{R}^2)_{ww}^{1/2}$ Å ^(a) Light scattering	$(\bar{R}^2)_{ww}^{1/2}$ Å				
		Kirkwood- Riseman	Kuhn- Kuhn	Flory- Fox	Debye- Bueche- Brinkman	Peterlin
C 2	1450	1300	1470	1405	1095	1450
C 8	1335	1140	1290	1210	950	1270
C 9	1150	1010	1145	1060	830	1125
C 10	775	620	705	610	480	695

^(a) Calculated from $(\bar{R}^2)_z^{1/2}$.

Table 21 b. End-to-end distances from sedimentation-diffusion data compared with the corresponding values from light scattering measurements.

Fraction	$(\overline{R^2})_{ww}^{1/2}$ Å (a) Light scattering	$(\overline{R^2})_{ww}^{1/2}$ Å				Peterlin
		Kirkwood-Riseman	Kuhn-Kuhn	Flory-Mandelkern	Debye-Bueche-Brinkman	
C 2	1450	1110	780	1060	625	—
C 8	1335	970	685	940	555	—
C 9	1150	860	605	810	480	—
C 10	775	530	375	490	285	—

(a) Calculated from $(\overline{R^2})_z^{1/2}$.

where the symbols are as previously defined. Values of $(\overline{R^2})_{ww}^{1/2}$ are given in Tables 20 and 21. The dimensions from viscosity data are in good agreement with those calculated from light scattering, but those from sedimentation-diffusion somewhat lower.

Mandelkern and Flory also introduced the following expression

$$\frac{s_0[\eta]^{1/3}}{M^{2/3}} = \frac{(\Phi^{1/3}P^{-1})(1 - V\rho)}{N_A\eta_0}, \quad (72)$$

where the left-hand side was said to be a constant and reported an average value of $(\Phi^{1/3}P^{-1}) = 2.5 \times 10^6$. Values of $(\Phi^{1/3}P^{-1})$ were calculated using the sedimentation data in equation (72) for the hydroxyethyl cellulose fractions and are included in Table 20. The values tend to decrease with decreasing molecular weight. Both Φ and P are known to be affected by the heterogeneity of the sample and, with cellulose derivatives, known to depend on molecular weight. However, the values are significantly greater than 2.5×10^6 .

Discussion

The hydrodynamic theories considered in this section all basically assume the configuration of the molecule to be represented by Gaussian statistics according to equation (47). Strictly speaking, random statistics apply to few polymer molecules in dilute solution. In the first place the configuration depends on the valency angle between successive monomer units and to steric and energetic hindrances about the bonds linking them; these are the short-range interactions. Secondly, the configuration is influenced by the volume requirement of the monomer unit, and thermodynamic interactions between any two monomer units and between the monomer and the surrounding medium; these are referred to as long-range interactions. An excellent review of this subject has been recently published by Peterlin [63]. It should also be noted that the various theories are only strictly applicable to monodisperse systems, which is rarely the case. It is well known that for flexible linear macromolecules the theories considered here give a reasonable interpretation of data at temperatures in the vicinity of the θ -point, where such molecules approximate to

the Gaussian model. Kurata *et al.* [61 *a, b*] and Ptitsyn and Eizner [62] have recently reviewed the theory of the excluded volume and shown that, in the neighbourhood of the θ -point, deviations from Gaussian statistics can be represented by a series expansion. However, even at temperatures removed from the θ -point, flexible chain molecules can be considered as a first approximation to be randomly coiling at all but the lowest degrees of polymerization. At present we thus have a good theoretical description of the behaviour of many flexible linear polymers in dilute solution, as their behaviour is not too removed from that predicted theoretically, and from viscosity and sedimentation-diffusion data one may successfully calculate average dimensions.

For cellulose derivatives, with usually experimentally inaccessible θ -points and having stiff, highly extended molecules up to very high degrees of polymerization, the theories may be expected to give an unsatisfactory interpretation of the experimental data owing to the pronounced deviation of the configuration from random statistics. Average dimensions for hydroxyethyl cellulose, calculated using the various relationships pertaining to the hydrodynamic theories, are presented in Table 21 together with the corresponding values of $(R^2)_{ww}^{1/2}$ calculated from the light scattering data for comparison. It is seen in Table 21*a* that the intrinsic viscosity data provide values of the R.M.S. end-to-end distance in excellent agreement with the values from light scattering. The corresponding R.M.S. end-to-end distances obtained from the frictional coefficient (Table 21*b*) are in each case substantially lower than those from the viscosity and light scattering data. This is a discrepancy that has been previously noted (see, for example, Kuhn, Kuhn, and Silberberg [55] and Peterlin [60]). For ethyl hydroxyethyl cellulose, Manley [11] calculated the R.M.S. end-to-end distances only from sedimentation-diffusion data. When the dimensions are also calculated from the viscosity data for his samples, a similar discrepancy to the above is apparent. Insufficient measurements of both intrinsic viscosity and sedimentation or diffusion have as yet been made on a large variety of polymers to reveal whether these deviations are partly due to basic weaknesses in the treatment of the frictional coefficient in addition to the molecule not strictly behaving as a Gaussian coil. However, it should be noted that the sedimentation-diffusion data give a reasonable value for the hydrodynamic radius of the monomer unit (Kirkwood-Riseman theory) and the hydrodynamic thickness of the molecule (Kuhn-Kuhn theory). The viscosity data and sedimentation-diffusion data thus provide supplementary information on the molecule. The Kirkwood-Riseman theory and the Flory-Fox theory give the least diverging values of $(R^2)_{ww}^{1/2}$ from viscosity data on one hand and sedimentation-diffusion data on the other.

As seen from the high values of the effective bond length, b , (Table 17) and equivalent chain segment length, A_m , (Table 19) from the viscosity data, the high extension of the hydroxyethyl cellulose molecule causes its non-Gaussian nature. The high extension of the chain, particularly evident at low degrees of polymerization, may be interpreted as being due to its limited flexibility owing to hindrances to rotation about the bonds linking the anhydroglucose units, caused by the large side groups. However, in the present case it is seen from the good agreement between $(\bar{R}^2)^{1/2}$ from light scattering and $[\eta]$ that the non-Gaussian behaviour of the molecule does not restrict the applicability of the viscosity theories described in this section. On the contrary, it gives confidence in their use even for such stiff molecules. The pronounced disagreement between $(\bar{R}^2)^{1/2}$ obtained from $[\eta]$ and s_0 (D_0) can perhaps be explained by the use in the frictional theories of relationships depending greatly on the permeability of the molecular coil. In the present case, the unexpectedly low

permeability (equations (24) and (25)) is probably due to the presence of the large side groups rather than tight coiling of the polymer chain. As $[\eta]$ is mainly dependent on the effective volume of the molecule, i.e. $(\bar{R}^2)^{3/2}$, and to a lesser degree on the permeability of the coil, the viscosity expressions are able to yield satisfactory values of $(\bar{R}^2)^{1/2}$. On the other hand, s_0 (D_0) is greatly influenced by the permeability of the coil and to a smaller extent by its overall dimensions. Thus the use of relationships between s_0 (D_0) and $\bar{Z}$ leads to erroneous results when calculating $(\bar{R}^2)^{1/2}$ because the low permeability of the present molecule depends on a factor other than the tight coiling. For example, in the Debye–Beuche theory the low permeability as reflected in the exponent of the frictional coefficient, ε_2 , predicts a tightly coiled molecule and consequently a small $(\bar{R}^2)^{1/2}$. However, the molecule has been shown to be well extended and the low permeability thus most likely arises from the extensive side groups. From this reasoning we see that the theories based on the frictional coefficient considering the dimensions of the coil in terms of relationships depending primarily on the permeability must lead to an underestimation of $(\bar{R}^2)^{1/2}$ when side groups of a bulky nature are attached. On the other hand these frictional theories are able to give a realistic picture of the internal structure of the coil, e.g. chain thickness and radius of the monomer unit.

SUMMARY

This paper deals with a non-ionic water-soluble cellulose ether, hydroxyethyl cellulose ($M.S. = 1.67$) and its characterization from a study of its macromolecular properties in dilute solution.

Hydroxyethyl cellulose was fractionally precipitated from water solution by the stepwise addition of acetone, producing fractions primarily with respect to the degree of polymerization. Eleven fractions were obtained by a two-stage procedure with the substantial elimination of high and low molecular weight "tails" (e.g. Fig. 2). Four fractions covering the molecular weight range of the unfractionated polymer were selected for the measurements described in this paper.

The distribution of substituent groups in hydroxyethyl cellulose is briefly discussed. A method is described for calculating the statistical distribution of the substituent attached directly to the anhydroglucose unit and that present as polyethylene oxide side chains (Fig. 4).

Viscosity measurements were made at different temperatures and different rates of shear. The shear dependences of relative viscosity (Figs 5 and 6) and intrinsic viscosity (Figs 8 and 9) were examined. Huggins' constant, k' , was found to increase linearly with intrinsic viscosity (Fig. 10) and decrease linearly with temperature (Fig. 11). The intrinsic viscosities showed a strong negative dependence on temperature (Fig. 12). Activation energies of viscous flow were calculated and the dependence on molecular weight determined (Fig. 16).

Sedimentation coefficients were determined, the values at infinite dilution ranging from 2.8 to 5.7×10^{-13} (Table 5). Integral distribution curves of sedimentation coefficients were plotted (Fig. 2) and provide a comparative measure of the heterogeneities of the fractions. Measurements of diffusion coefficients yielded values at infinite dilution between 0.8 and 1.9×10^{-7} cm²sec⁻¹ (Table 7). The partial specific volume was found to be 0.701 (Table 8). Molecular weights, $\bar{M}_{ww}$, calculated from the Svedberg equation lay in the range 125,000 to 545,000 (Table 9). Relationships between $[\eta]$, s , D , and $\bar{M}_{ww}$ were established (equations (23), (24) and (25)).

Number average molecular weights, $\bar{M}_n$, were determined by osmotic measurements and lay between 125,000 and 380,000 (Table 10). The second virial coefficients, A_2 , showed an insignificant variation with molecular weight and had the approximate value of 11×10^{-4} ml mole/g². By measuring A_2 at a series of temperatures, the θ -point was found to be at 200°K (Fig. 27).

The refractive index increment, (dn/dc) , was determined as 0.139 ml/g at 546 m μ and 0.141 ml/g

at 436 $m\mu$ (Table 12). Using the Dandliker procedure of directly centrifuging the light scattering cell, it has been possible to obtain consistent data on aqueous solutions of hydroxyethyl cellulose. Weight average molecular weights, M_w , lay between 190,000 and 625,000, the corresponding z -average radii of gyration between 450 and 750 Å, and the second virial coefficients between 3.9 and 8.5×10^{-4} ml mole/g² (Table 13). The polydispersities of the fractions, M_w/M_n , lay in the range 1.5 to 1.8 (Table 14). The Flory-Fox parameter, Φ' , was found to increase with increasing molecular weight towards the asymptotic limit of 3.2×10^{22} found for flexible polymers. The molecular expansion factor, α , was found to be effectively constant, the values lying between 1.02 and 1.04. The effective bond length, b , decreased with increasing molecular weight from 33 to 29 Å (Table 15). For the highest molecular weight fraction the Porod-Kratky persistence length, q , was 83 Å. The steric hindrance parameter, $\cos \phi$, was 0.89. It is concluded that the hydroxyethyl cellulose molecule is highly extended at low molecular weight, but at high molecular weight is extensively coiled and its configuration approaches that of a randomly coiled polymer molecule. In addition, the presence of the substituent groups makes the hydroxyethyl cellulose molecule significantly stiffer than the cellulose molecule.

The viscosity and sedimentation-diffusion data are considered in the light of recent hydrodynamic theories of macromolecules developed for ideal systems. The viscosity data provide end-to-end distances, $(R^2)_{ww}^{1/2}$, in good agreement with those calculated from light scattering data, while the sedimentation-diffusion data give substantially lower values in all cases (Table 21). This discrepancy is probably caused by the use in the frictional theories of relationships depending greatly on the permeability of the molecular coil. In the present case, the unexpectedly low permeability is probably due to the presence of the large side groups rather than tight coiling of the polymer chain, leading to an underestimation of $(R^2)^{1/2}$ from s_0 (D_0).

ACKNOWLEDGEMENTS

The investigation described in this paper was carried out at the Institute of Physical Chemistry of the University of Uppsala during the years 1958–1961.

I wish to express my sincere gratitude to the head of the Institute, Professor Stig Claesson, for introducing me to this field, for his continuous interest and valuable encouragement and advice and for placing the excellent facilities of the Institute at my disposal.

My sincere thanks are due to Dr. David Henley for his encouraging interest and stimulating discussions during this work and to Mrs. Anita Säflund for her friendliness and frequently needed assistance.

I should also like to express my warmest thanks to my colleagues at the Institute for their friendship and the many ways in which they have facilitated this investigation.

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Tryckt den 27 oktober 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

Errata

Page 240, Fig. 10:

The ordinate scale should read: from 0.0 to 1.0 *instead of* 0.0 to 10.0.

Page 242, text to Fig. 14 should read as follows:

Logarithm viscosity in *centipoises* plotted against reciprocal absolute temperature for hydroxyethyl cellulose fraction C9 in water.

Studien über den Effekt von Wirkstoffen an grünen Pflanzen und an Tumoren

VON HANS V. EULER, BETH V. EULER und HANS HASSELQUIST

I. Wurzelbildung

Seit einer Reihe von Jahren werden in diesem Institut Versuche angestellt, durch welche die an transplantierten Tumoren fungierenden Ergone verglichen werden sollen mit gewissen in Pflanzen tätigen Wuchsstoffen.

Versuche mit Urethanen, die nach Haddow und Sexton [1] ebenso wie andere Substanzen (K. H. Bauer [2]) an Ratten sowohl cancerogen als auch anti-cancerogen auftreten können, ergaben an Blattstielen von *Impatiens Balsamina* zunächst keine signifikanten Änderung der Wurzelbildung [3].¹

Dass die Wurzelbildung stofflich beeinflusst werden kann ist seit längerer Zeit bekannt; die Art und Stärke dieser Beeinflussung, besonders die chemische Natur der dabei wirksamen Faktoren wurde in mehreren Arbeitskreisen eingehend studiert, wozu seit 1932 Kögls Arbeiten über Auxine und besonders seine Entdeckung des Heteroauxins und dessen Wirkung vielfache Anregung gaben.

Was die Stimulierung der Entwicklung grüner Pflanzen (F. W. Went [4], K. V. Thimann [5], F. Laibach [6], Zimmermann [7]) und besonders die Wurzelbildung an Blattstielen und Stengeln betrifft, so sind aus neuerer Zeit in erster Linie die Untersuchungen von Kurt Mothes und seiner Mitarbeiter [8] zu nennen.

Orientierende Versuche über Wurzelbildung an Stengeln verschiedener Pflanzen

Die in Lösungen verschiedener Stoffe eintauchenden blattragenden Sprossen von 5-cm Länge wurden jungen Pflanzen entnommen durch Abschneiden dicht unter einem Knoten.

A. Impatiens Balsamina

Die bekannte Förderung der Wurzelbildung durch Heteroauxin (β -Indolylessigsäure) schrieb F. W. Went einem spezifischen, von ihm *Rhizokalin* benannten Phytohormon zu, das aber bis jetzt nicht genügend gekennzeichnet ist. Schon bei unseren früheren Untersuchungen hatte sich gezeigt, dass zur Beeinflussung der Wurzelbildung höhere Konzentrationen von Heteroauxin erforderlich sind als zur Zellstreckung. Wie bei der Cancerogenese in Tieren hat man bei der Wurzelbildung

¹ Den Mittelwert des pH-Optimums der Wurzelbildung kann man zu 5,5 annehmen.

Tabelle 1. Redukton-Derivate und N-Oxyde.

Substanz	Konzentration	Vers.zeit Tage	Resultat
Redukton-Anthranilsäure, pH 6,0	2 mg/100 ml	8	Keine Einwirkung.
	10 mg/100 ml	14	Auf kräftigem Spross kleine nicht wachsende Wurzeln. Auf jüngeren Spross Zerstörung des Stengels.
Red.- <i>m</i> -nitro-anil	10 mg/100 ml	7	Wurzelbildung 2 Tage verzögert. Wurzelzuwachs normal.
Red.- <i>o</i> -oxy-anil	10 mg/100 ml	9	Vollständige Hemmung d. Wurzelbildung.
Red.-di-anthranilsäure	5 mg/100 ml	5	Keine wesentliche Hemmung, aber 4 Tage Verzögerung. d. Wurzelbild.
4-Nitro-chinolin- <i>N</i> -oxyd pH 6,5	10 mg/100 ml	9	} Keine Wurzelbildung.
	5 mg/100 ml	7	
	2 mg/100 ml	10	
Chinolin- <i>N</i> -oxyd, pH 6	5 mg/100 ml	13	Wurzelbild. verzögert. Nach 13 Tagen Wurzeln nur 0,5–3 mm.
Diäthyl-nitrosamin pH 5,5	400 mg/100 ml	6	Vollständige Hemmung, Blätter fallen ab.
	200 mg/100 ml	10	Starke Verzögerung.
	20 mg/100 ml	10	} Keine Einwirkung. Wurzelbild. wie im Wasser.
	4 mg/100 ml		
Isopropyliden-adenosin pH 6,5	2 mg/100 ml	12	Wurzeln 25 mm; Kontrollen 16 mm.

Tabelle 2.

Vers.	Nukleinsäuren	Konzentration	Vers.zeit Tage	Mittlere Wurzellänge	
				in Vers. Lös.	in Wasser-Kontr.
I.	Desoxyribonukleinsäure	10 mg/100 ml	18	40 mm	12 mm
		20 mg/100 ml	9	30 mm	10 mm
II.	Desoxyribonukleinsäure	20 mg/100 ml	7	2 mm	1 mm
			9	15 mm	6 mm
			11	21 mm	8 mm
	Ribonukleinsäure	15 mg/100 ml	9	8 mm	2 mm
	KH ₂ PO ₄ pH 6,5	15 mg/100 ml	8	3 mm	1 mm
			12	25 mm	11 mm
	KH ₂ PO ₄ pH 6,5	5 mg/100 ml	12	im Vergleich mit DNS ¹ ; die Wurzeln gleich gut entwickelt.	

durch Phytohormone zwei verschiedene Wirkungen zu unterscheiden: Die Initialwirkung und die Wachstumswirkung. Die Mindest-Konzentration des Heteroauxin-Beitrages zur Wurzelbildung finden wir bei 1–3 mg/1000 ml.

¹ 20 mg/100 ml.

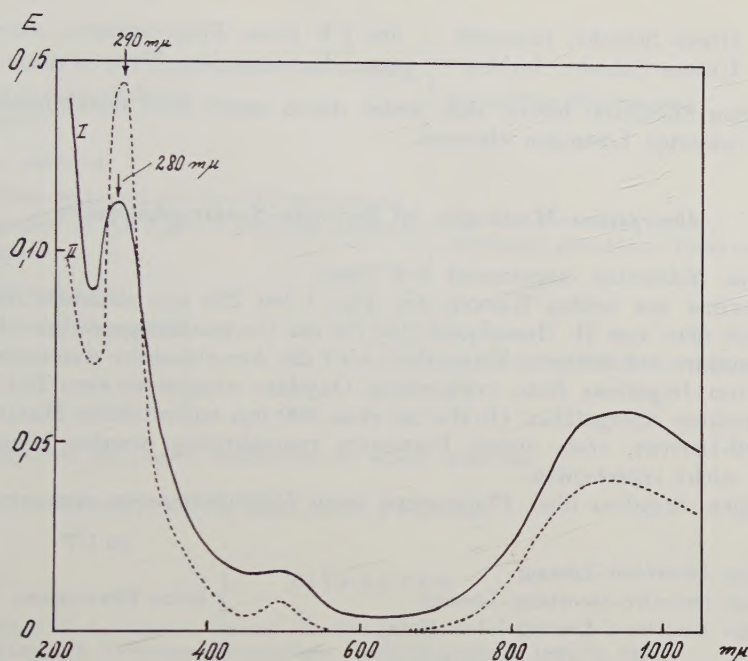


Fig. 1.

Lösung I: 0,5 %ige Ascorbinsäure-Lösung.

Lösung II: 0,5 %ige Ascorbinsäure-Lösung + H_2O_2 .

Die an einzelnen Sprösslingen erhaltenen Werte der Wurzellängen variieren je nach dem Zustand der Mutterpflanze. Die Wirkung der DNS und RNS dürfte wesentlich mit ihren Phosphat-Gruppen zusammenhängen und durch die Nukleasen der Sprossen [9] vermittelt werden.

II. Messungen an Lösungen verschiedener Substanzen nach der Reaktion mit Sprossen (H. Hasselquist)

Entsprechend der S. 1 angegebenen Problemstellung handelt es sich hier um die Kennzeichnung der in Wasser bzw. in Ergonlösungen während der Wurzelbildung auftretenden Faktoren. Ihre Mengen waren bei unseren orientierenden Versuchen so gering, dass an eine Ausfällung und Umkristallisierung nicht gedacht werden konnte, sondern optische bzw. chromatographische Methoden zu Hilfe genommen werden mussten.

Beobachtungen und Messungen an *Impatiens Balsamina*.

Was zuerst an den 0,5 %igen Ascorbinsäure-Lösungen nach 5–6-tägigem Eintauchen der *Impatiens*-Sprossen auffiel, war die Gelbfärbung, die sich innerhalb einer weiteren Woche in Rotbraun vertiefte. Im UV fluoresziert diese Lösung stark.

Wir wollten die farbigen Komponenten der genannten Lösung trennen, und zwar durch Adsorption an aufgeschlämtem Al_2O_3 in einer engen Chromatogrammröhre. Nach der Adsorption wurde mit 500 ml Wasser gewaschen. Es zeigten sich dann deutlich 2 Schichten:

Obere Schicht, braunrot	im UV keine Fluoreszenz
Untere Schicht, farblos	im UV blauweisse Fluoreszenz.

Die beiden Schichten liessen sich weder durch saure noch durch neutrale oder alkalische wässrige Lösungen eluieren.

Absorptions-Messungen im Beckman-Spektrophotometer

Impatiens Balsamina eingetaucht 5–6 Tage.

Die Maxima der beiden Kurven der Fig. 1 bei 290 m μ stimmen recht nahe überein mit dem von H. Hasselquist [10] für ein Umwandlungsprodukt der Dehydroascorbinsäure ermittelten. Vermutlich wird die Ascorbinsäure durch eine in den Sprossen von *Impatiens Bals.* vorhandene Oxydase wenigstens zum Teil in Dehydroascorbinsäure übergeführt. Ob die bei etwa 500 m μ auftretenden Maxima einem Chlorophyll-Derivat, etwa einem Porphyrin zugeschrieben werden können, lässt sich noch nicht entscheiden.

Die obigen Angaben über Fluoreszenz seien folgendermassen ergänzt:

	im UV
0,5 %ige Ascorbins.-Lösung	} keine Fluoreszenz
0,5 %ige Dehydro-ascorbins.-Lösung	
0,5 %ige Ascorbins.-Lösung + 1 % H ₂ O ₂	
Sprossen in 0,5 %iger Ascorbins.-Lösung	} kräftige Fluoreszenz
Sprossen in 0,5 %iger Ascorbins.-Lösung + 1 % H ₂ O ₂	
Sprossen in Wasser	schwächere Fluoreszenz

Frühere Versuche mit Kobalt-Salzen an *Impatiens Balsamina* (H. v. Euler, *Arkiv Kemi* 1, S. 488 (1949) waren mit Sprossen angestellt worden, die vor der Kobalt-Behandlung bereits 15 mm lange Wurzeln besaßen. Durch die Co-Molaritäten 0,02, 0,01 wurde ein Welken der Blätter der Sprossen hervorgerufen; neue Blätter traten in etwa 1 Woche nicht auf.

Auch in neueren Versuchen wurden zu hohe Co-Konzentrationen angewandt: in Lösungen von 13 mg CoCl₂/100 ml trat eine Zerstörung des Stengels ein.

Endoxan aktiviert. Iso-propyriden-adenosin hat in 8 Tagen an *Impatiens Balsamina* in Konzentrationen von 10 mg/100 ml keine Wurzelbildung, also Hemmung im Vergleich mit Wasser hervorgerufen. Die Versuche gehen weiter.

Eine auffallende Erscheinung wurde an *Impatiens* während der Wurzelbildung in Wasser und in Ascorbinsäure-Lösung, die in Glasgefässen standen, beobachtet: von etwa dem 3. Versuchstag an wendete sich der Stengel der Sprossen in der Lösung nach oben (also gegen die Wasseroberfläche zu). Es trat also ein Tropismus ein, den wir einstweilen noch nicht identifizieren können.

B. Neue Serie

Coleus pumilus Blanco, Labiatae

Setcreasea purpurea B. K. Boom, Commelinaceae

Hibiscus rosa-sinensis L., Malvaceae

Zebrina pendula Schnitzl, Commelinaceae

Setcreasea purpurea

Sprossen in 0,5 %iger Ascorbins.-Lösung	}	grünblaue Fluoreszenz
Heteroauxin-Lösung		
Wasser		

Coleus pumilus

7 Tage während starker Wurzelbildung	}	schwache grünblaue Fluoreszenz
Sprossen in 0,5 %iger Ascorbins.-Lösung		
Wasser		

Zebrina pendula

während Wurzelbildung	
Wasser	schwache grüne Fluoreszenz

Herrn Ingenieur Alf Larsen danken wir für eifrige Mitarbeit.

Stockholm, Universität, Inst. für organ.-chem. Forschung.

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Tryckt den 27 oktober 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

